

Progress on chemical weapons treaty

The decision of the United States House of Representatives to fall in with the Pentagon's request for funds to manufacture chemical weapons again is a serious, even a sombre, development. For the best part of a decade, both the Administration and Congress have been more concerned with the disposal of chemical weapons than their production. From time to time, the US Army has excited public interest and anger by its often clumsy arrangements for shipping unwanted chemical munitions about the place, with the consequence that the dumping of useless weapons at sea has been superseded by more rational techniques for the chemical decomposition of the active ingredients. Although the munitions disposed of by these means have usually been unserviceable or even unsafe, the mere act of disposal has chimed in well with the widespread belief that preparations for chemical warfare would serve no useful purpose, and might even damage the discussions between the Soviet Union and the United States about the form that might be taken by an international convention to prohibit chemical weapons. To be sure, the Pentagon has made no secret of its ambitions to get back into the production business, but Congress (the paymaster) has been stolidly unwilling to agree. Now, it appears, all this has changed (see page 100). Last week the House of Representatives agreed to provide the US Army with the funds needed to clear a site in Arkansas. In the next few weeks, it is almost certain that the appropriation for a pilot plant to manufacture binary chemical munitions will follow. Why has there been such a turnabout?

The influences on the US Congress are plain enough. Especially in an election year, few congressmen will wish to stand out against the Department of Defense. It would be different if detente had not collapsed, or been eroded, by the events in Asia in the past six months. Reports of the Soviet use of chemical weapons in Afghanistan, however insubstantial they may be, must tend also to undermine the resolve of Congress. Yet there must be a more intricate explanation of the sudden flurry of interest in chemical warfare, which is not confined to the United States. Last week, in Britain, a group of Conservative MPs gave it as their view that British cities are especially vulnerable to attack by chemical weapons. In doing so, they amplified some of the concern that members of the British government have expressed about Soviet intentions and the inconclusiveness of the Soviet-American discussions towards the draft of a treaty. What can be the explanation?

The erosion of detente has understandably set people's nerves on edge, but strategic developments in Europe have no doubt also helped to stimulate the current preoccupation with chemical weapons. The West German Chancellor, Mr Helmut Schmidt, makes no secret of his anxiety about the increasing numbers of medium-range missiles on the territory of the Warsaw Pact, not yet matched by the deployment of American cruise missiles within NATO. Yet there is no chance of an agreement to limit these developments while Salt II remains unratified. Until quite recently, European opinion would not have been excessively alarmed by this imbalance. While it has been customary (as it is prudent) to speculate on the ways in which short and medium-range weapons might be used in anger within the confines of Europe, people have usually been willing to accept that the strategic forces of NATO would be used if there were more than token trouble. The novel development is that this confidence has also been eroded, largely as a result of the hesitancy of American

foreign policy in recent months. Are these also the circumstances which have led to the speculations that gas might be used in Europe without provoking a strategic response?

If so, it would be helpful that the strategic limitations of chemical weapons should be kept clearly in mind. Whatever the technical sophistication of modern chemical weapons, their strategic limitations remain what they have always been. It is unthinkable that the use or the threatened use of chemical weapons could be used to win a decisive advantage in some major conflict — or that an attempt to use them in this way by any state would fail to excite a nuclear response. The technical utility of chemical weapons, such as it may be, lies in the battlefield — and the Second World War shows that the fear of retaliation may be enough to engender restraint. The possibility that chemical weapons might be used in a nuclear war to win some larger advantage, perhaps that of making it safe to occupy some large tract of ground without first blowing it to pieces, is more real, yet still remote. It is hard to see even the most determined combatants trading the occupation of some other state's territory for the destruction of their own. In short, chemical weapons cannot by themselves substantially shift the balance of strategic strength in any major conflict, which is not to say that the military may not regard them as potentially useful in other kinds of conflicts.

So why not get rid of them? This question has been in the air for the past decade or so. Since 1974, the Soviet Union and the United States have been trying to work out the bare bones of an agreement on the subject. So far, there have been no fewer than eleven rounds of talks on the subject (and the twelfth is now going on at Geneva). A report on progress was presented to the Committee on Disarmament, and another is promised for later this year. While the past six months have seen a slackening of interest, some progress has been made. Yet the problems recognized as serious at the beginning persist.

Verification, as always, is a stumbling block, especially because plants for manufacturing the components of binary chemical munitions may be indistinguishable from ordinary chemical plants. Last year's report on the negotiations used the term "adequate verification" and suggested an international secretariat to supervise the way in which verification would be carried out. It is, however, hard to see the most significant potential signatories of a treaty to ban chemical weapons accepting the degree of detailed inspection that their potential adversaries could require. Any treaty that comes about will therefore demand of its participants a degree of trust in each other (as well as in their sources of intelligence) that has not been much in evidence in the past few years. Yet the closely analogous convention on biological weapons has survived even last year's outbreak of anthrax in the neighbourhood of Sverdlovsk. A convention on chemical weapons could be drafted along similar lines — for which reason it would be a great benefit if the Soviet Union were to provide a more detailed account of the anthrax outbreak than it has so far provided.

The scope of a treaty banning chemical weapons is another of the issues with which people have been wrestling for the past several years. Here again it is hard to see how a fully watertight treaty could ever be drafted. Some materials used in the manufacture of nerve gases have a legitimate place in the chemical industry (while phosgene is widely used as a chemical intermediate). The expressed intention of the Soviet Union and



the United States of using incapacitating gases for domestic riot control is another serious snag. Here again, however, there is no reason why even the major powers should not accept a certain degree of fuzziness in what might be required of fellow signatories. It is not as if a violation of whatever treaty there might be would give the violator a decisive advantage.

So what are the prospects that a treaty will eventually be negotiated? And to what extent will the course of events be influenced by the willingness of the House of Representatives to let the Department of Defense have its pilot plant? Given that the Soviet Union (but also France) has the means of using chemical weapons tactically, and on the assumption that, even if the American pilot plant were followed by a full-scale production plant, the munitions produced would be similarly earmarked, there is no reason why the talks at Geneva should be interrupted. Indeed, the new development may persuade the Soviet Union that it would be prudent to make faster progress.

And there is little doubt that, if they chose to do so, the major powers could agree on the text of a treaty that would satisfy their own interests. They have learned, in the past several years, that it is possible to live with measures of arms control whose terms are not as specific as meticulous lawyers would ask. So the prospects for the treaty must depend on whether (and if so when) the major powers decide that the time has come to edge back towards detente. Many will hope that they take the plunge sooner rather than later. When that time comes, they will encounter one of the other longstanding problems of arms control — the unwillingness of other states to sign on somebody else's dotted line. On chemical weapons, France and China at least are likely to insist on their freedom to do what they do at present. Especially now that France has rejoined the enlarged Committee on Disarmament, has not the time come when the major powers should surrender the initiative to the committee? No harm, but only good, could come of such a step.

Two views on technological change

How will new technology affect the structure of society? Off and on, for the past few decades, people have been brooding on this kind of issue. This week, it became known that the British Labour Party is cautiously on the side of the pessimists. An unpublished report due to be considered by the party's National Executive later in the month correctly concludes that recent development in the technology of microprocessors will mean even more unemployment in the United Kingdom unless economic growth keeps pace with increases of productivity now foreseen; predictably, it goes on to argue that increased state intervention in the management of technical and social change is needed. This view neatly follows the publication by the OECD of a report called "Science and Technology in the new socio-economic context" prepared by what the OECD calls a "Group of Experts" for the longstanding Committee for Scientific and Technological Policy (see page 98). The OECD document comes to the same conclusion as the Labour Party, but if anything its prescription is more a charter for interference by social scientists than by governments.

During the past three years, there has been a more or less constant grumble that the applications of microprocessors now in prospect have serious consequences for people's jobs. Some will change in character. Some will disappear altogether. These calculations are correct, although the timing is as ever unsure. It does not follow, however, that microprocessors mean mass unemployment. With this as with any technological innovation, improved productivity in one important sector of industry will mean increased wealth, increased demand for goods and services and therefore more jobs in other sectors. The steam engine, whatever the Luddites said and did about it, was as dramatic a source of increased productivity as ever microprocessors will be — and the end result, at least in countries where steam engines were allowed to make a mark, was more jobs. The only reason not to welcome the coming of the microprocessors is that in some places the potential benefits of their increased productivity will not materialize, or will not be matched by comparable increases of productivity stemming from other new technologies.

The Labour Party document, to its credit, has the wit to recognize that this must be the case. The issue that will be disputed is whether more government intervention is the best way of safeguarding the future of jobs in Britain. The dispute is partly ideological. The Labour Party believes in government intervention. The present government does not. Since neither has a monopoly of wisdom, the need is to decide what governments can and cannot do. Providing every classroom or at least each school with a microcomputer might provide a sporting chance that people leaving school were familiar with one new technology, but is this the wisest course when the state school system is crying out for funds with which to buy books, mathematics text-books among them? Equally, the Department of Industry's scheme for helping industrialists become familiar with the new technology,

useful though it may be to some of the participants, is no substitute for investors' sense that they can profitably invest in the British economy, and industrialists' sense that the taxation system will allow them to keep some of the profits. In short, what governments can do is to manage the climate for innovation. Governments themselves are less good at innovation. (This is no excuse for the continued delay in deciding whether in Britain the Department of Industry is to let the microprocessor company INMOS have its second £25 million; the government has a moral obligation that will not go away with the passage of time.)

Broadly speaking, these are the conclusions of the OECD report. The committee marshals impressive evidence which gives the lie to the cynical view that research and development is irrelevant to industrial innovation. The correlation between industrial (as distinct from government) spending on research and development and eventual prosperity is strong. But which is the chicken and which the egg? The OECD committee, while recognizing the difficulty, puts the issue beyond doubt. Governments wishing to foster innovation must foster research and development by industry, but without falling back on short-term fiscal incentives. It is a hard and self-denying recipe, that would entail in Britain some attenuation of the government's own research enterprise. Yet the case for doing more, not less, when times are hard is undeniable.

On the reasons for the hiatus in the affairs of the industrialized countries in the past seven years, the OECD is nothing if not robust. When money is being drained out of the economy, largely by OPEC, cannot be the best time for innovation or even mere change. All that is easier when things are going well. Yet this is a turning point in many people's affairs. More productivity is in the long run the only way out of the crisis. Although the report is short on references to the economists' gurus, Keynes and Friedman alike, it leaves the clear impression that the committee that prepared it considered that innovation is not so much a problem as an answer to an unstated problem. The committee's dissident voice, Professor Emma Rothschild, found this escalator uncomfortable because she had no clear idea where it is going. That is too much to ask.

That is the sensible part of the OECD report. It is less sensible that much of the document is taken up with the supposed need of what is fashionably called technological forecasting. This, after all, is a discipline which has yet to demonstrate itself as such. Many will suspect the phrase to be yet another licence for social scientists to make contradictory assertions about the consequences of technological innovations. The trouble, of course, is that the consequences are almost always unpredictable. All that can confidently be said is that some consequences are profound. What societies faced with rapid technological change thus need of their governments is flexibility — a capacity (and a willingness) to meet unforeseen social changes with compassion and imagination.

Red faces (and hot tempers) on 2,4,5-T

The controversy over the use of the herbicide 2,4,5-T in the United Kingdom became even more heated last week with the revelation of a difference of opinion between government agencies over how to determine the safety of the herbicide. Thus Mr John Locke, the director-general of the Health and Safety Executive (HSE), pleaded for a more effective relationship between the executive and the Advisory Committee on Pesticides (PAC) of the Ministry of Agriculture, Fisheries and Food (MAFF).

Locke was speaking at the opening of a new HSE centre at the Royal Show (see page 100). His call for more "tying up" between HSE and PAC is, on the surface, a plea for more rationality in the review processes for toxic substances, but it underscores the strain both organizations are suffering in determining the safety or otherwise of 2,4,5-T.

The director-general's comments were voiced two days before a meeting of the HSE's Advisory Committee on Toxic Substances (ACTS), where 2,4,5-T was the main item on the agenda. Like all HSE advisory committees, ACTS has representatives from both the Trades Union Congress (TUC) and the Confederation of British Industries (CBI). With the TUC already on record as calling for a ban on 2,4,5-T, feelings were bound to run high. By all accounts it was a stormy meeting, with the TUC representatives expressing their dissatisfaction with PAC's review processes for herbicides and pesticides.

As a general rule, the TUC's representatives on ACTS believe that, where issues of health and safety are concerned, HSE should be responsible. The unions suspect the impartiality of MAFF in reviewing the toxicity of chemicals which can be of benefit to agriculture. At the meeting last week, they asked why PAC claimed in its eighth review of 2,4,5-T (published in March last year) that only three tonnes of the herbicide were used annually in Britain, when the real figure is now known to be about twenty times as much. They also want to know the names of all UK manufacturers importing, exporting or selling products containing 2,4,5-T.

Recent Customs and Excise figures confirm that some 116 tonnes of 2,4,5-T were imported into the UK in 1979. With the publication of these revised estimates, the unions are all the more sceptical of the review processes.

MAFF is seriously embarrassed by the error about the tonnage of 2,4,5-T used in Britain. PAC had assumed — wrongly — that the Forestry Commission was the largest user of the herbicide in the UK, and the three tonne figure was based on 2,4,5-T use in silviculture. It is now known that far more 2,4,5-T is used on grassland; on the

basis of a recent survey, the use of 2,4,5-T in Britain last year is now put at 58 tonnes.

One especially serious aspect of the affair is that the PAC estimates were given to the Royal Commission on Environmental Pollution during its inquiry into agricultural pollution. In the report of this inquiry published last year, the royal commission gave 2,4,5-T a clean bill of health, partly on the grounds that British usage was relatively small. Members of the commission were wondering this week how the record should be set straight.

PAC is now reviewing the herbicide for the ninth time in the light of information about 2,4,5-T and its dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) contaminant published in the past two years.

The subcommittee responsible, on the instructions of Mr Peter Walker, Minister of Agriculture, has now met a delegation from the National Union of Agricultural and Allied Workers (NUAAW) to hear the union's case against 2,4,5-T. In three and a half hours of often heated discussion, the union delegation insisted that 2,4,5-T should be banned. The delegation was told that the dioxin content of 2,4,5-T now set at a maximum of 0.01 parts per million in the UK is so low that it represents no threat. The herbicide itself is a teratogen in some rodent species at concentrations of 35 milligrams per kilogram of body weight, 10,000 times the concentrations at which

dioxin is a teratogen. With the new 2,4,5-T formulation, 2,4,5-T will exert a teratogenic effect before dioxin.

The subcommittee also believes that there is a sufficient safety factor in the assessment of the carcinogenic risks from dioxin. The most reliable study suggests that dioxin is a carcinogen at dose levels of 0.1 microgram per kilogram of body weight per day in rodents. With the new formulation, to consume such a quantity of dioxin, rodents would need to take in 2,4,5-T at concentrations about one-tenth to a half of their body weight.

The subcommittee's recommendations on 2,4,5-T will be considered at a PAC meeting on 24 July. The same evidence will also be reviewed at a special meeting of ACTS at the end of August. Whatever the outcome of these meetings, it is clear that the trade union campaign against 2,4,5-T has had a considerable effect on HSE and MAFF.

The unions see the visit of the NUAAW deputation to the PAC scientific subcommittee as a precedent. But it is unlikely that the trades unions will be satisfied with this modest advance. The unions are openly campaigning for a change in the review process, and it was only natural that John Locke should appeal to MAFF for some help in devising a system which will satisfy all parties.

Alastair Hay

Supreme Court reprieves benzene

Washington

Strict regulation of toxic substances must be backed up by adequate scientific data on their potential danger. This was the message delivered by the US Supreme Court last week when it rejected a proposal by the Department of Labor's Occupational Safety and Health Administration (OSHA) to reduce permitted exposure levels to benzene to one part per million.

Following evidence which emerged in the early 1970s of a link between benzene exposure and various blood disorders — in particular, leukaemia — OSHA produced new regulations in 1977 which would have reduced permitted exposure levels from the current 10 parts per million.

The proposed regulation has been attacked by the chemical and petroleum industries, where more than a million workers are exposed to benzene in products from gasoline additives to paint solvents. The industries claimed that the regulations were unnecessarily restrictive; and that the cost of meeting them — measured in hundreds of millions of dollars — was incommensurate with the expected benefits.

Using such arguments, the American Petroleum Institute last year persuaded a

judge in New Orleans to rule the proposed regulation invalid. Much attention had been focused on OSHA's decision to appeal to the Supreme Court, largely because the judge criticized OSHA for not weighing the expected benefits of the regulations against the costs of meeting them.

In the event, the Supreme Court skirted around the cost-benefit issue. Only one member — Justice Lewis Powell — insisted that a full cost-benefit analysis should have been carried out; and the matter awaits resolution in two further cases which the court has agreed to hear next year.

No less significant, however, is the substance of the court's ruling in a case which Chief Justice Warren Burger described as raising "difficult unanswered questions on the frontiers of science and medicine". The court ruled that OSHA must demonstrate that a toxic chemical poses a "significant" risk before making a regulation.

This judgement directly undermines one of the fundamental principles on which OSHA's regulatory strategy is at present based. So far, the agency has argued that, where a chemical is suspected of having low-level toxic effects but the scientific and epidemiological evidence is insufficient to characterize these precisely, it must be

assumed that there is no threshold below which the chemical can be considered "safe" — and industry is required to lower exposure to the limits of technological feasibility.

OSHA acted on benzene even though the evidence of increased leukaemia incidence among workers in Turkey and Ohio involved people who had been exposed to considerably more than 10 ppm over long periods of time.

The agency argued that only a technological feasibility strategy would suffice to guarantee the protection of workers, but the Supreme Court, in a 5-4 vote, agreed with industry critics that a more thorough scientific and technological justification must be produced. Admitting the existence of considerable uncertainties, the court accepted that the agency is not required to prove its point "with anything approaching scientific certainty".

Even so, in writing the majority opinion, Justice John Stevens said that OSHA had failed to produce "substantial evidence" that the new benzene rules were "reasonably necessary and appropriate" to remedy a "significant risk of material health impairment". OSHA, he said, had not produced empirical evidence or scientific opinion to show that exposure to benzene at or below the 10 ppm level had in fact caused cancer.

Predictably, the court's decision has been welcomed by industry, which has long protested at the expense of meeting the "lowest technologically feasible" criterion. Agency officials admitted that they were "disappointed" with the ruling, but vowed to press on with regulating benzene and other toxic substances.

The closeness of the Supreme Court's decision and the fact that different members gave different reasons for concurring are likely to cushion the impact. Indeed, if OSHA can produce more evidence on benzene, then the tough standard could still stand.

In a strongly worded dissenting opinion, four of the nine justices said they felt OSHA had already produced enough evidence to justify the low exposure limit. And a fifth said that, given additional evidence, he might be persuaded by OSHA's case.

David Dickson.

Soviet environment

Spending more

SOVIET capital investment in environmental protection and the conservation of endangered species will be trebled in the next Five Year Plan (1981-1985), according to what TASS has described as "preliminary data". Since the official figures for the present plan include 11,000 million rubles of direct state

funding and 50,000 million from the budgets of industrial enterprises and institutions, this suggests an investment of at least 180,000 million rubles (£125,000 million) in the next five years.

The TASS announcement followed the passing of two major environmental bills, on clean air and the protection of fauna, by the Supreme Soviet. Enactment of the laws was preceded by unusually extensive reporting in *Pravda*, perhaps because of the undoubted pride which the members of the Supreme Soviet show in their progress to date. No other country, observed Deputy P.P. Vavilov, president of the All-Union Academy of Agricultural Sciences, has on its statute books laws of comparable scope, which even protect insects and soil fauna with a direct bearing on soil fertility.

Vavilov nevertheless emphasized that, even when the act becomes law in 1981, further research will be needed to ensure that its provisions are implemented in strict accordance with ecological principles.

What nobody mentioned in the debate, but what must surely have been in the minds of many of the deputies — especially those from central Russia, central Asia, Byelorussia and Estonia — was the present threat from wolves and wolf-dog cross-breeds which, according to some estimates, have doubled in numbers over the past ten years, following cutbacks in hunting and the disbanding of skilled hunting teams. The threat is particularly acute in Estonia.

No less complex are the management problems raised by the clean air bill. Air pollution has been a major problem in many Soviet cities for a considerable time, and one of the earliest preparations for the Moscow Olympic Games was the designing of electric runabout cars for the Olympic village, to reduce the effect of petrol fumes

on the athletes.

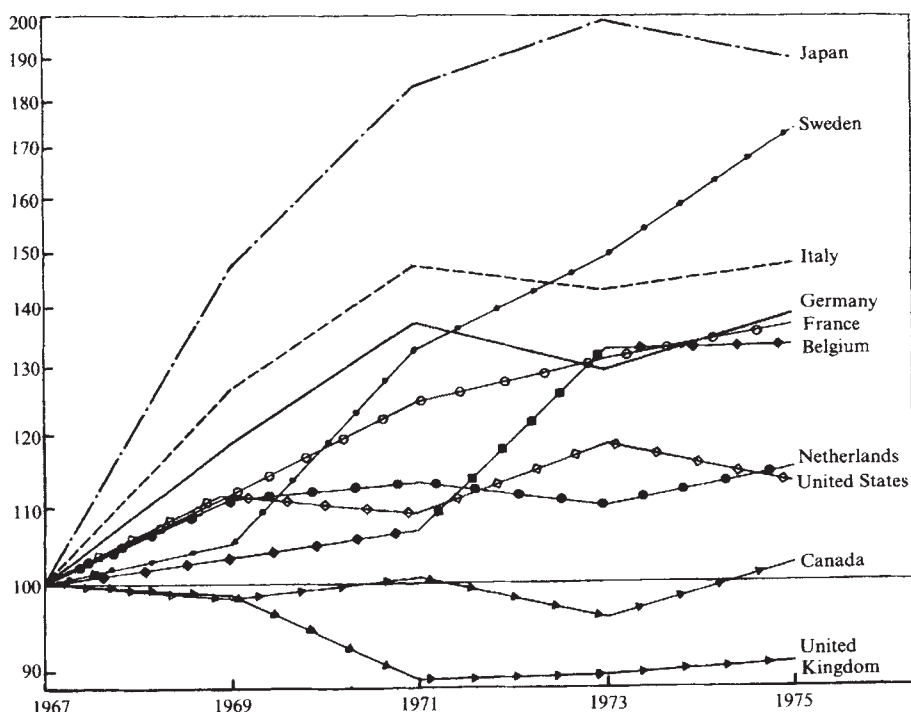
Not only cities are at risk, however. In the Supreme Soviet debate, Deputy V.M. Kanun from Zhitomir spoke of the pollution threat to the Polesian forests — in spite of the expenditure, during the current Five Year Plan, of "tens of millions of rubles" on anti-pollution measures. Deputy Yu. A. Izrael', Chairman of the State Committee on Hydrometeorology and Environmental Monitoring, observed that the Union-wide anti-pollution monitoring service now has air-pollution monitoring projects in 450 cities; nevertheless, as Deputy Kipel' observed, "many plants and factories go on polluting the atmosphere and water in the same old way".

Water, in fact, is not specifically the subject of the new legislation, although, according to Deputy Izrael', progress in this field is one of the "major successes" of the environmental control programme. "Almost all the rivers of the European part of the USSR", he said, "have become cleaner".

Nevertheless, water may well soon be better regulated. A 200-person multidisciplinary research team, led by Vladimir Lazanskii, head of the All-Union Research Institute for Water Conservation, has completed a detailed computer analysis of the ecology of 26 of the largest rivers in the USSR and fifteen seas and lakes. On the basis of this study, the team has put forward several proposals ranging from the automated control of water conservation complexes and the introduction of closed-cycle processes for metallurgical works to the special processing of rain water and snow melt bearing particles of petroleum products.

Vera Rich

Industrially financed research, OECD countries



Biotechnology

France now

A GROUP of French scientists has set up a commercial organization for the exploitation of genetic manipulation. Known as *Transgène*, the company has an initial capital of 40 million French francs. Although the capital has been provided by industrial companies, the sponsors of the new venture include the CNRS (Centre National de la Recherche Scientifique), L'Institut National de la Recherche Agronomique, L'Institut National de la Santé et de la Recherche Médicale and the Pasteur Institute.

The new organization will be based at its own laboratories near Strasbourg and its policy will be determined by a scientific committee including Professor Philippe Kourilsky from the Pasteur Institute and Professor Pierre Chambon from the Louis Pasteur University of Strasbourg. It is intended that the scientific committee will include some scientists from outside France. The scientific director of the enterprise has been recruited but not yet named.

It is understood, however, that the director is at present the head of research at a European pharmaceutical company and is not French.

The formation of *Transgène* has been talked of for several months. The capital for the enterprise has been provided by a consortium of companies organized by the Compagnie Financière de Paris et des Pays Bas (Paribas) and includes the oil company Elf, the French liquid air company Air Liquide and the champagne and brandy firm of Moët-Hennessy. The commercial head of the new enterprise will be the director of research at Paribas, M. Robert Lattès. It appears that this French initiative has been prompted — some would say provoked — by the publicity given in the past few months to the doings of the Swiss-

based company Biogen, but the organizers of *Transgène* also have in mind the steps taken in the United States during the past few years to put genetic manipulation on a commercial footing. Although nothing is known as yet of the work on which *Transgène* will be engaged, it is expected that the company will be recruiting a staff of between 50 and 80 people, most of whom will be based at the laboratories in Strasbourg.

Transgène appears to have hit on a novel way of reconciling the conflict of interest between university researchers and commercial interests that inevitably arises in such circumstances. Fifteen per cent of the shares are to be held by the sponsors of the enterprise, including the Pasteur Institute and the CNRS. A further eight per cent of the shares will be the property of a foundation yet to be established whose function will be to support basic research in the general field of molecular biology — a task that will become feasible if and when *Transgène* makes money.

The Strasbourg laboratory will not be completed for two years. In the meantime *Transgène* is hoping to make arrangements for research to begin on a contract basis in some other laboratory, possibly at the Louis Pasteur University. The scientific staff will be recruited in time to begin work at the turn of the year but the initial scientific objectives will not be defined until the scientific board is complete around October.

University industry

Surrey consults

SHORTAGES of funds are increasingly making British universities search for alternative sources of finance. The University of Surrey has in this way branched out beyond the usual pattern of university work. Last week it formally opened a new institute to tackle industrial health and safety problems.

Surrey's Institute of Industrial and Environmental Health and Safety has actually been operating for 18 months. So far, it has earned about £700,000, one-third from grants and donations and the rest from specific contracts. According to its director, Professor James Bridges, the institute is already almost entirely self-financing and hopes eventually to make a profit which can be ploughed back into the university. The members of the institute's staff are either tenured in an academic department, on short-term research council contracts or have been specifically employed by the institute on annual rolling contracts.

The institute is an amalgamation of several different academic units (the Wolfson Bionalytical Unit, the Materials Handling Research Unit, the Industrial Biomedical Unit and the Toxicology Unit). It also includes two new units, the Isolator (Gnotobiotics) Unit and the Occupational Health and Hygiene Service. The last of these is the most unexpected. It carries out no research as such, but aims to provide local industries too small to employ their own specialists with advice on health and safety problems.

The Occupational Health and Hygiene Service appears to be an effective way of making links with local industry. An investigation of sore throats and bronchitis at one local factory led to the Wolfson Bioanalytical Unit being called in to take air samples. It finally identified the source of irritation as chromium carried from one part of the plant to another when the wind was in a particular direction. The Wolfson Unit also solved a problem in the handling of glass fibre resins in a local factory after a similar introduction.

Most of the institute's work so far has been funded by larger organizations. The Materials Handling Unit, for example, has completed an ergonomic study for the EEC of the body stresses caused when men lift heavy objects, and is now doing a similar study of female workers for the UK Health and Safety Executive.

The Isolator Unit was created to build up on a previous collaboration with Vickers Medical Limited to design and make medical isolators out of flexible film. The unit is now designing film units for isolating experimental animals and protecting workers handling drugs and other potentially dangerous materials. The Toxicology Unit is engaged on fundamental work on biochemical pathways in man and animals, but most of its contract work consists of *in vitro* testing for mutagenesis and carcinogenesis.

The institute acts more as a research association than a university research department. According to its staff, the prospects for the next year or two are good, with plenty of work on hand. Thereafter, the institute will have to live on its wits, helped by whatever reputation it acquires in the meantime.

Judy Redfearn

Poles protest at academic censors

SCIENTIFIC publications should be totally exempt from state censorship, according to a report by the Polish "DiP" group (*Doswiadczenie i Przyszlosc* — Experience and the Future). This unofficial body, which includes leading figures from the academic and literary professions, Party and church, was originally established in November 1978 as a discussion group to consider the economic crises facing Poland. Unable to hold the open discussions they had planned, the members of DiP have relied on questionnaires. The first such survey, "Report on the state of the country and ways of putting it right", was made in May 1979; the second, "How do we get out of this?" in December 1979 and January 1980. This second report is now in circulation.

Questionnaires were completed by 141 people. (Some of those approached declined to take part saying that they had no faith in the sense of the exercise.) The sample included 65 scientists, of whom 39 were university lecturers and professors — biologists, physicists, mathematicians and agronomists. The result is a penetrating study of the economic and social problems of the Polish People's Republics.

A necessary condition for any reforms to be successful, it says, is that confidence should be restored in the government. To this end, *inter alia*, a free flow of information is essential. A proper definition of material liable to censorship (military and industrial secrets) should be laid down, and academic publications exempted from the censors.

Chemical weapons

Congress agrees

Washington

Citing circumstantial evidence of the use of poison gas by Soviet troops in Afghanistan, the US Congress has taken the first step towards ending an eleven-year moratorium on the production of chemical weapons.

By-passing the White House — which has nevertheless so far raised no objection — the House of Representatives last week approved the army's use of funds to renovate facilities at its Pine Bluff Arsenal in Arkansas to house the production of so-called binary chemical weapons.

The amount of money is relatively small — \$3.1 million out of a total military construction budget of \$4,800 million. A further \$19 million will be needed to equip an intended pilot plant for the production of 155 mm binary shells. Given minimal opposition, either from within Congress or from the Administration, legislators pushing for the pilot plant are confident they can persuade the House to provide the extra cash, and that the Senate will go along. If so, the Pine Bluff plant could be in operation by 1983.

The Department of Defense has planned for two further extensions of the plant at a cost of \$185 million — production plants for 8-inch artillery shells containing the British nerve agent VX and for so-called "big eye" bombs for the US Navy, like the 155 mm shells containing the nerve gas GB.

Binary weapons, the latest thing in the military gas-man's catalogue, offer safe storage and use; two relatively innocuous chemical components which react to produce a military gas only after the weapon has been primed. The US Army has been working on their development since the mid-1960s.

In 1974, Congress turned down a proposal to manufacture them, on the grounds that the Soviet-American negotiation of a treaty covering chemical weapons, begun that year, would be jeopardized.

Officially, those talks still drag on. In each of the past two years, the Department of Defense has asked Congress for funds for binary weapons production, pleading Soviet superiority in both offence and defence. On each occasion, the request has been blocked by the White House.

Now the political climate has changed. Although Presidents Carter and Brezhnev agreed last year in Moscow to encourage the negotiations, the Soviet presence in Afghanistan has brought progress to a standstill. The future of the treaty, some US officials consider, will now depend on broad political considerations and not on the question whether or not the United States is producing chemical weapons.

Reports of the Soviet use of chemical agents in Afghanistan helped to change the

mood of Congress and the Administration, although the details are still far from clear. US officials say there is now "strong" evidence of the use of riot-control agents, "middling" evidence of incapacitating agents, but that evidence of the use of lethal nerve agents is "weak". They support a proposal for an international fact-finding mission to gather more precise details of events in Afghanistan.

These reports, combined with accusations of Soviet development of biological weapons after the anthrax outbreak in Sverdlovsk, have created a climate in which support for a unilateral moratorium is waning. Some critics nevertheless remain vocal. Chemical weapons are of little real tactical value, "contributing nothing to fire power unless the other side chooses to use chemical first" says Professor Matthew Meselson of Harvard University.

In Congress, however, those who have previously resisted the chemical weapons programme have no plans to organize opposition to the new developments. Some argue that, since the army's existing stock of wet-eye nerve bombs is deteriorating and could become a serious health hazard, the production of binary weapons would be the lesser of two evils.

The White House occupies neutral ground. It has not supported the congressional amendments to its defence request, but neither has it opposed them. In the present climate, if Congress does give the green light to the chemical weapons facility, a presidential veto is unlikely.

David Dickson

British farming

Science on show

Agricultural shows still have the same appeal for the British public as they did in Thomas Hardy's day. The Royal Agricultural Society of England's annual show, however, is more than family entertainment. The theme of last week's show, like that of recent years, was the application of science in agriculture. Publicly supported research was much in evidence.

The Ministry of Agriculture, Fisheries and Food (MAFF) has always prided itself on the value of its research and development to the agricultural industry. In the wake of the Rothschild reorganization, almost half of the work done by the institutes of the Agricultural Research Council (ARC) is under contract from government departments. This year, for example, MAFF is spending about £34 million on basic research, matched by a similar contribution from the ARC. About £2 million will come from other sources. The Agricultural Development and Advisory Service (ADAS) will spend £54 million on getting the results of research to the farmer. The work of ADAS and ARC institutes was therefore prominent at the

Royal Show. No doubt in an attempt to stave off the criticism that much of this research is too basic to bear on farmer's practical problems, the exhibits included examples of both pure and applied research.

The National Vegetable Research Station (NURS) displayed its basic research on the introduction of resistance to tobacco mosaic virus into tomato plants. It is also seeking to interest British food processors and growers in varieties of small outdoor tomato plants which yield fruit that is superior to (although more expensive than) Italian canned tomatoes.

Import substitution is a recurrent theme. The NVRS, proud of having helped to reduce the level of British onion imports since the early 1970s by the introduction of Japanese varieties which can be planted all the year round, is now working towards the home-grown baked bean.

On animal nutrition, the Rowett Research Institute is improving on the starch equivalent as a means of assessing the metabolic value of feeding stuffs. The Animal Diseases Research Association was last week offering advice on the prevention of hypothermia in lambs and the Meat Research Institute on the measurement of fat deposition in live animals. The long-term objective is to find better ways of controlling the proportion of fat to lean meat.

In these and other ways, the Royal Show has become an annual celebration of the notion that British agriculture is outstandingly efficient. The increase of agricultural productivity in the past 30 years is certainly impressive. The belief that British agriculture is the most efficient in the world has become almost a myth, difficult to challenge.

Such a challenge has come, however, from the Centre for Agricultural Strategy at the University of Reading. In a report published last week, the centre says that British agriculture is by no means streets ahead of European agriculture. Belgium, the Netherlands and Denmark may be doing better. The centre argues that British farmers have invested too much capital in their businesses, especially in farm machinery. The Royal Show last week would have tempted them further down this primrose path.

Judy Redfearn

Fusion

Studied design

Planning time is running out for the INTOR project, the international design study of a thermonuclear reactor set up last year by the International Atomic Energy Agency. At the ten-day biennial conference on fusion and plasma physics just ended in Brussels, the members of the international design team (Euratom, Japan, the Soviet Union and the United States) confirmed that their final report will be with the IAEA next June. Then, it is acknowledged, will be the time for tackling

the political problems.

INTOR stands for International Tokamak Reactor. The objective so far has been to define the design parameters of a 620 MW (thermal) tokamak which would test engineering systems required in a commercial fusion reactor. The objectives of INTOR are to produce a token amount of electricity and to demonstrate tritium breeding and 25-50 per cent availability. Decisions on the future of the project will be taken by members of the design team only when the final report is ready.

For the time being, the team is innocent of politics. The first difficult step will come when the design study is complete and it is necessary to set up a full-time design group.

If ever built, INTOR will be a net consumer of electricity and tritium. The reactor would use a charge of 3 kg of tritium which would have to be replaced every six months. Average electrical power consumption would be 200 MW. Capital costs would be of the order of the cost of the American Engineering Test Facility, about \$1,000 million. **Robert Walgate**

US fusion

Middle course?

Washington

Magnetic fusion research in the United States will be further encouraged by a new report prepared for the Department of Energy. The report concludes that research is now sufficiently encouraging to justify a new stage of engineering experimentation.

Even so, the report falls short of endorsing the Engineering Test Facility being promoted by some members of the fusion research community. It says that the proposed high neutron flux and heavy usage, as well as the role of the ETF as a direct stepping-stone to commercial fusion, are "too ambitious" and "inappropriate at this stage".

The report recommends instead that the department should construct, at an estimated cost of less than \$1,000 million over ten years, a more modest Fusion Engineering Device (FED) based on the tokamak design. This device, the report says, should be sited at a new Center for Fusion Engineering (CFE).

The proposals come from a subcommittee of the department's Energy Research Advisory Board, chaired by Dr Solomon Buchsbaum of Bell Laboratories. Officially they are still in draft form; but they are likely to form the backbone of the Administration's response to critics who claim it has been dragging its feet on the commercial application of fusion.

The Buchsbaum committee in passing attacks a previous report prepared two years ago by a similar committee chaired by Dr John Foster of TRW Industries. That argued that it would be unwise to pursue an ambitious programme based on the tokamak design, urging that research

support should be spread more widely.

The Administration has responded by increasing support for alternative designs, in particular for the Elmo Bumpy Torus being developed at the Oak Ridge National Laboratories (*Nature*, 1 May), to the chagrin of the potential commercial sponsors of fusion technology, who have felt that the Foster committee was too cautious.

Leading the attack has been Mr Mike McCormack, chairman of the energy subcommittee of the House Science and Technology Committee. Mr McCormack has been pushing hard for an accelerated fusion research and development programme. Last year his subcommittee appointed a 12-member Fusion Advisory Panel which recommended that the ETF, being designed at Oak Ridge, should be built in 1987.

The Buchsbaum committee is the response to the McCormack initiative by Dr Edward Frieman, director of the Office of Energy Research. It justifies its shift of view by citing recent "rapid scientific progress" and says that the US is now ready to embark on the exploration of the engineering feasibility of fusion.

Among recent successes are:

- validation of empirical predictions of achievable electron energy confinement times;
- demonstration in experiments with neutral beam currents that the heating of plasma to ignition temperature is feasible;
- the absence of evidence that impurities will tend to accumulate in the centre of high density plasmas; and
- the existence of several promising schemes for steady-state current drive using radio-frequency or particle-injection techniques.

These and other developments, the committee says, make it appropriate to move on to the study of ignited plasmas — where fusion reactions proceed without external energy being supplied. This would be one of the principal goals of the FED, which would also be used to explore reactor technology and to study safety questions. But the new report, like that of the Foster committee, asks that attention should continue to be given to alternative designs in case the tokamak configuration disappoints.

The committee therefore supports the proposal for a large tandem mirror machine, if only to prove the principle that open-confinement systems may be feasible; this may be included in the 1982 budget request. The committee also asks that work on the Elmo Bumpy Torus should be strengthened to clarify some immediate physics issues, but adds that the proposed \$75 million investment is "too large, given the existing uncertainties in the physics of the EBT configuration".

The appearance of the committee's draft report aptly coincided with a debate in the House of Representatives on the Department of Energy's proposed 1981

budget. As part of a general attempt to trim long-range energy research, the House Appropriations Committee had proposed that the magnetic fusion budget be reduced by \$23 million from the Administration's request for \$396 million (an 11.5 per cent increase over this year).

Waving copies of the Buchsbaum report, House members were able to restore the money by an amendment which also reduced some of the proposed cuts in high-energy and nuclear physics research programmes. But Mr McCormack is not satisfied. He is now talking of introducing a supplementary budget request later in the year to bring the fusion budget more into line with the Buchsbaum proposals, which recommend that the total budget be almost doubled over the next five to seven years.

Not unexpectedly, the Buchsbaum report is less bullish than Mr McCormack. It says that heavy spending on the engineering aspects of fusion will not be necessary until 1983-84. By then, results from the Tokamak Fusion Test Reactor at Princeton will be available, and should help to decide the design details of the proposed Fusion Engineering Device.

David Dickson

New NSF director

Dr John B. Slaughter (below) was nominated to take over from Richard C. Atkinson as director of the National Science Foundation last week. Slaughter, an engineer by training, has been persuaded to return to the NSF after leaving only a year ago to become academic vice-president and provost of Washington State University. He had previously been assistant director of NSF for astronomical, atmospheric, earth and ocean sciences. Atkinson, a psychologist, has already taken up an appointment at the University of California.



Albanian science

Isolated still

Albania is embarking on its own characteristically isolated programme of science and technology. At the two-day plenary meeting of the Central Committee of the Albanian Workers' Party last month, the main theme was the need to increase the level of scientific research and to apply research and development more effectively for the development of the country.

The issue is timely. The five-year plan (1981-85) now being prepared includes increases of industrial and agricultural production of 40-42 per cent. But with the departure of the Chinese, "self-reliance" has become the order of the day. Addressing the plenum, Ramiz Alia of the Party's Political Bureau paid a glowing tribute to the Albanian "specialists,

technicians and workers" who had had to take over the various technological projects left unfinished when the Chinese quit.

According to Alia, Albania is now able to design and set up "self-reliantly" every object the economy needs, whether "industrial, hydrotechnical, railway or cultural". Self-reliance is also to be the watchword for the future. In the natural sciences, he stressed the need for research in applied nuclear physics and the mastering of neutron and laser technology for both industrial and military use. Research projects in biophysics should also, he said, be started.

On the applied side, Alia emphasized the importance of water management; he said that 52 per cent of the arable land in Albania is now irrigated — the highest proportion in Europe. Alia also called for strains of wheat capable of producing 70 to 80 quintals per hectare, an increase of 10 to 15 per cent compared with existing hybrids.

Alia said that fundamental work was needed to reach the target of 95 per cent extraction of useful minerals.

The first Secretary of the Central Committee of Albania, Enver Hoxha, in his closing address to the plenary session, put the Party's demand in more general terms. Albania, he said, has now reached an advanced stage of economic and social development, so that what is now required of science is "a generalization, improvement and an entire positive transformation in practice and theory — a qualitative break, a revolution in production technique, technology or organization". Technological advance, however, must not be accompanied by a drift from agriculture. Development of the agricultural sciences and the intensification of agricultural production is aimed at maintaining the current demographic distribution, in which some two-thirds of the Albanian population live in the countryside.

Vera Rich

CORRESPONDENCE

Cruel Malthus

SIR, — Peter Laslett¹ does injustice, I believe, to British social science in stating that, except perhaps for John Maynard Keynes, "Thomas Robert Malthus may be quite justifiably called . . . the most important of all English-speaking social scientists". From the "population principle" which Malthus claimed² to have "established in the first six pages" of his 1798 *Essay*, Malthus concluded that poverty was caused primarily by the "vices" of the poor themselves.

Accordingly, "to assist the poor in such a manner as to enable them to marry as early as they please and rear up large families is a physical impossibility³". Ferociously consistent, this mild-mannered cleric and professor advocated abolition of the poor laws which sheltered, though inadequately, the helpless poor. He opposed repeal of the corn laws, universal suffrage, and a regularly meeting representative parliament.

Malthus's stature as a social scientist may be judged from his pronouncement⁴ that "The structure of society in its great features will probably always remain unchanged. We have every reason to believe that it will always consist of a class of proprietors and a class of labourers." Yet his doctrine, which was born during the French Revolution, has surfaced repeatedly in various guises at times of social stress.

This recurrence is not particularly surprising. Malthus himself stated the political utility of his "principle" quite baldly in later editions of the *Essay*⁵.

"Among the poor themselves, its effects would be still more important. That the principal and most permanent cause of poverty has little or no direct relation to forms of government or the unequal division of property; and that as the rich do not in reality possess the power of finding employment and maintenance for the poor, the poor cannot in

the nature of things possess the right to demand them, are important truths flowing from the principle of population which when properly explained would by no means be above the most ordinary comprehension.

"And it is evident that every man in the lower classes of society who became acquainted with these truths would be disposed to bear the distresses in which he might be involved with more patience; would feel less discontent and irritation at the government and the higher classes of society on account of his poverty; would be on all occasions less disposed to insubordination and turbulence; and if he received assistance either from any public institution or from the hand of private charity, he would receive it with more thankfulness, and more justly appreciate its value.

"If these truths were by degrees more generally known . . . the lower classes of people as a body would become more peaceable and orderly, would be less inclined to tumultuous proceedings in seasons of scarcity, and would at all times be less influenced by inflammatory and seditious publications from knowing how little the price of labour and the means of supporting a family depend upon a revolution.

"The mere knowledge of these truths, even if they did not operate sufficiently to produce any marked change in the prudential habits of the poor with regard to marriage, would still have a most beneficial effect on their conduct in a political light; and undoubtedly one of the most valuable of these effects would be the power that would result to the higher and middle classes of society of gradually improving their governments without the apprehension of those revolutionary excesses, the fear of which at present threatens to deprive Europe even of that degree of liberty which she had before experienced to be practicable, and the salutary effects of which she had long enjoyed."

While Malthus's hopes for pacifying the "poorer classes" have not been conspicuously successful, the doctrine has found a new use in the post-war years, as the poor in the developing countries have raised demands on their erstwhile rulers. The Malthusian resurgence⁶ assuages the conscience of the "class of proprietors" in the rich countries and facilitates inaction by their governments.

Yours faithfully,

HARRY GRUNDFEST

College of Physicians and Surgeons,
Columbia University,
New York, NY, USA.

Nuclear protest

SIR, — Your leading article of 19 June concerning nuclear power exemplified many of the elitist opinions of the scientific "community". It appears you would like the public to reach an understanding of "the niceties of nuclear power" similar to its understanding of the relation between salary rises and inflation. The simplistic view of the economy (salary rises cause inflation) that many people have been impressed on them by successive governments and the Tory press. Is this how you want the nuclear power debate to proceed?

Your arrogant suggestion that the willingness of people to demonstrate is sustained only by the division of "professional" opinion is insulting to the many demonstrators who have arrived at an anti-nuclear position after careful consideration of available information on all the aspects of nuclear power.

The "key to the future of nuclear power" does not belong with a small and self-interested clique of professionals; it belongs with society as a whole. The real trouble with the nuclear debate is not that it is too polite but that it centres on technical discussion while ignoring the political, for example, the proliferation of nuclear weapons that is already accompanying the proliferation of nuclear power, the setting up of an armed secret police force and the denial of trade union rights (which is one of the main political reasons for developing nuclear power, according to Thatcher). Hopefully, members of the public will continue to see through the "leave it to the experts" myth.

Yours faithfully,

BRIAN CUMMINS
ALAN BEARE
DAVE SMITH

Edinburgh Science for People,
Biochemistry Department,
University of Edinburgh, UK.

1. *Nature*, 285, 173 (1980).

2. 6th Ed., 2, 453.

3. 7th Ed., 479 (1872).

4. *ibid.*, 480.

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NEWS AND VIEWS

How to use geothermal energy

from S.W. Richardson & A.A.L. White

WITHIN the next decade it will be possible to obtain large quantities of hot water from permeable geological formations buried deep in the sedimentary basins of the UK¹. The temperature of the water is restricted by the terrestrial heat flow and the occurrence of suitable formations to a maximum of 110°C (ref. 2) but this limit could be extended if the technology is developed to extract heat from impermeable crystalline rocks³.

There is a multitude of possible uses for this relatively low grade heat but here we consider the relative merits of just two such applications: the generation of electricity and the provision of district heating. These uses are very different in structure. District heating is, at first sight, the obvious application — and has dominated official thinking for several years⁴ — but is capital intensive due to the cost of the network of pipes needed for distribution. The plant required to convert the heat into electricity is relatively cheap but provides a much less efficient use of the energy due to the low conversion efficiencies of heat engines operating at these low temperatures. To illustrate the differences between these schemes we investigate the case of two hypothetical entrepreneurs, one considering investing in boreholes and a distribution network hoping to make a profit by selling heat to householders; the other aiming to sell geothermally generated power to the electricity utility.

It is reasonable to assume that the scheme which offers the higher return on capital is more likely to be implemented. Costs and income for converting a small city to district heating are found using figures produced for the Combined Heat and Power reports⁵⁻⁷. Electrical generation is provided by 10 MWe plant based on the Rankine cycle with an organic working fluid and costs are taken from American generic studies^{8,9}. The geothermal energy is assumed to be provided by pairs of production-reinjection boreholes or 'doublets' sunk into proven aquifers at 3 km depth containing water at 100°C. Aquifer transmissivity is taken to be $3.5 \times 10^{-4} \text{ m}^2 \text{ s}^{-1}$ and flow rates of 50 kg s^{-1} are maintained by pumping. The cost of providing these holes⁸ is £1.0M per doublet for a scheme involving a number of doublets.

District heating

Allowing for heat exchanger effectiveness and losses in the distribution system, we assume that each doublet will be able to provide water at 90°C to the householder. With a 60°C (ref. 5) return temperature this represents some 6.3 MWth, sufficient to provide for 1520 households (see p.85 of ref. 5). The cost of the distribution network varies with the housing density^{5,7} and the capital costs of this scheme are as follows.

Household density per hectare	12	20	50	150
Heat load, MW km ⁻²	6	10	25	75
Distribution pipework cost per household £ (1980) (ref 5, p 80)	2600	1270	600	520
Total costs per doublet £M (1980)	4.95	2.93	1.91	1.79

It has been assumed that £1 (1976) = £1.6 (1980) and that all the boreholes are drilled within the city (extra significant figures are included allowing the argument to be followed in detail).

The energy papers found that each household will require 39 GJ per year⁶ so the installation of the network will allow the entrepreneur to sell $5.9 \times 10^{13} \text{ J}$ per year.

The sale price of this heat will be determined by the price of alternative fuels since householders will not choose to convert their existing wet central heating system, or pay to have a number of radiators installed, unless the heat is considerably cheaper: indeed a major obstacle to district heating could be the need to coerce people to join the system⁷. However, in this work we have assumed 100% penetration of the heat market as this was the figure used to derive the costs in the energy papers. The current UK prices for heating alternatives, making due allowance for the inefficiency of domestic boilers, are shown below. Geothermal district heat sold at each of these prices produces the annual income shown.

Against income must be set the pumping

A.A.L. White is a Research Officer at the Central Electricity Generating Board's Marchwood Engineering Laboratories. S.W. Richardson, a Lecturer in Geology and Fellow of St. Edmund Hall Oxford, is currently on leave at Marchwood.

Fuel	Price, £ per GJ	Income, £ per year
Gas	1.95	116,000
Coal	2.40	142,000
Oil	5.50	325,000

costs since the 'doublet' operation will require some 250 kWe. At 38% load factor⁶ this represents £29,000 per yr. where the electricity is purchased from a utility at 3.5p per kWh. The net incomes are shown as percentages of the capital costs in the Figure and are seen to vary from 2% per yr. if the heat were supplied at gas prices to a sparse area of 12 households per hectare, to 16% if heat were supplied at oil prices to a densely populated area with heat demand from 150 households per hectare.

Obviously a geothermal district heat scheme will be relatively attractive in a fuel economy dominated at the present time by oil. This is the case in France and explains the energetic French exploitation of their geothermal resources in heating schemes. In Britain, where the heating market is dominated by gas, geothermal district heating schemes will be rather less attractive as an investment.

The income from each doublet may be increased if it is possible for the householders to accept a lower return water temperature of 50°C, allowing each borehole to supply 2,025 households. However, the network costs increase in proportion and the returns on capital are not significantly altered.

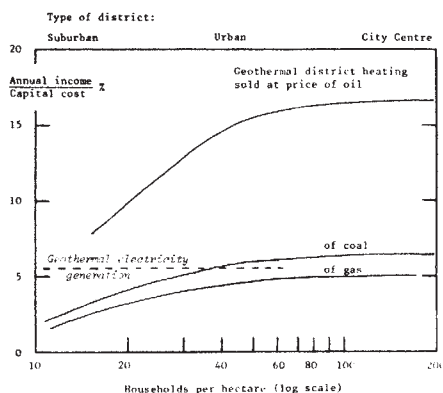
Similarly the load factor of the geothermal system could be increased by adding fossil fuel fired boilers to the network to provide for peak demands. However, the network extension required to accept the boreholes' output cause the improvements, if any, to be of second order.

Electricity generation

The efficiency of Rankine cycle generation from a hot water geothermal source is¹⁰

$$\eta = \alpha \left[1 - \frac{T_0}{T_2 - T_1} \right] \ln \left[\frac{T_2}{T_1} \right]$$

where T_0 is the heat rejection temperature determined by the environment, T_2 is the



How return on capital for a geothermal district heating scheme varies according to the household density and the equivalent fuel price.

geothermal source temperature and T_1 is the temperature at which the geothermal fluid is reinjected into the aquifer. α is an empirical constant which is determined by the losses in the power cycle and we take the value $\alpha = 0.6^8$. For $T_2 = 373$ K, $T_1 = 308$ K and $T_0 = 298$ K, $\eta = 0.073$ and the gross electrical output is 1 MW per doublet. The capital cost of the plant is £754,000⁸ and the cost of the boreholes and connections to the central plant is £1.34M per doublet. Total capital is thus £2.09M for a net electrical output of 750 kWe. Operating at an 80% load factor this entrepreneur is able to sell some 5.3×10^6 kWh each year to the utility worth £116,000 at the current price of 2.2p per kWh paid by the Electricity Council's Area Boards. The return on capital is 5.5% per year.

Relative and absolute merits of the applications

Of the two schemes, electricity generation would have a higher return on capital if the price at which the other sold heat were determined by gas, for all dwelling densities. For a density of 40 households per hectare it would require an increase in the price of gas by 30% with the electricity price unchanged for district heating to hold the advantage. On the other hand, if the price of heat were fixed by oil, district heating would provide the better investment for densities greater than 12 households per hectare. The relative merit of the schemes is not sensitive to the assumed temperature of the aquifer.

It should be noted that we have stacked the odds in favour of district heating, in three respects particularly. First, the boreholes are assumed to be within the district they serve. Heat transmission would materially raise the capital cost of the scheme. Second, we have included no

'overheads' allowance for the district heating scheme. For the electricity scheme most of these costs are included in the difference between the Area Board's buying and selling price for electrical energy. Third, we have assumed (with refs 5 and 7) 100% market penetration. To achieve this, district heating would in fact have to set its price considerably below that of competing fuels.

Neither of the schemes is particularly attractive in absolute terms; but should energy prices as a whole increase relative to other costs this particular conclusion could well be reversed. In a regime under which a

large proportion of electricity is generated from fossil fuels, major relative changes in electricity and fossil fuel prices seem unlikely.

We conclude that the generation of electricity from low grade heat is at least as likely to become an attractive proposition as the provision of geothermal district heating in countries which, like Britain, generate a large proportion of their electricity from fossil fuels. Paradoxically, the geothermal electricity option saves money but wastes fossil fuels: we suspect that none but the most high minded will be put off.

Changes in length-of-day and atmospheric circulation

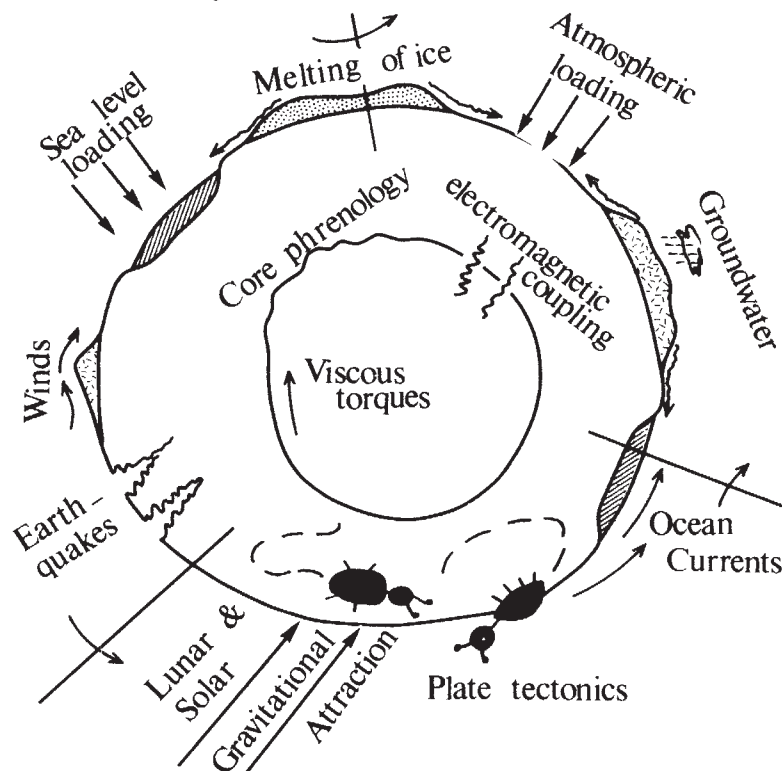
from Kurt Lambeck

OBSERVATIONS reported in this issue of *Nature* (Hide *et al.* p141) show that zonal components of atmospheric momentum contribute to high frequency changes in the length-of-day.

For many years astronomers have observed fluctuations in the Earth's rate of rotation — or changes in the length-of-day — that are attributable to various forces acting on the Earth's mantle and to a redistribution of mass within the Earth, oceans and atmosphere. Despite the smallness of these departures from

uniform rotation — changes in length-of-day of about 10^{-3} s typically occur over time intervals of a few days to several years, and changes of about 10^{-2} s occur over several decades — they have intrigued geophysicists for many years and a complete discussion requires one to delve into atmospheric and oceanic circulation problems, into the Earth's elasticity and anelasticity and into magnetohydrodynamics of the core^{1,2} (Fig. 1). Astronomers measure the integrated amount τ by which the Earth is slow or fast

Fig. 1 Schematic illustration of the forces that perturb the Earth's rotation. Topographic coupling of the mantle to the core has been referred to as 'Core Phrenology' by R.R. Hide while the beetles, following T. Gold, represent the effect of continental drift.



1. Oxburgh, E.R. *Nature* **262**, 526 (1976).
2. Oxburgh, E.R. *et al.* in *Advances in European Geothermal Research EECXII/185/80EN* Brussels (1980).
3. Batchelor, A.S. in *Advances in European Geothermal Research EECXII/185/80EN* Brussels (1980).
4. Garnish, J.D. *Energy Paper* no. 9 HMSO (1976).
5. Wright, J.K. *et al.* *Energy Paper* no. 20 HMSO (1977).
6. Cassels, J.M. *et al.* *Energy Paper* no. 34 HMSO (1979).
7. Marshall, W. *et al.* *Energy Paper* no. 35 HMSO (1979).
8. Milora, S.L. & Tester, J.M. *Geothermal Energy as a Source of Electric Power* (MIT Press, 1976).
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10. White, A.A.L. *Inst. Elect. Eng. Proc. A* (in the press).

after a certain time interval by comparison with a uniform time scale and the first derivative of this function gives a measure of the change in the length-of-day. Figure 2 illustrates the spectrum of the proportional changes in the length-of-day. Present techniques give accuracies for τ of about 1.5×10^{-3} s for an integration time of about 5 days³ but new observational techniques based on laser range measurements to artificial satellites or to the Moon and long-baseline radio-interferometry have been developed. Preliminary results^{4,5} indicate that improvements in resolution and precision are imminent.

We can anticipate that a new part of the length-of-day spectrum will rise above the measurement noise level (see Fig. 1). For example, rapid changes in length-of-day may occur in conjunction with very large earthquakes, similar to the Chandler wobble problem^{6,7}, in which the direction of the rotation axis is displaced relative to the Earth's crust when large earthquakes occur. Such changes will only be evident if there is no 'meteorological noise' in the length-of-day data or if the meteorological contribution to the length-of-day changes can be reliably evaluated from the meteorological data.

The contribution of the atmosphere to the broad spectrum of rotation perturbations has long been recognized as being important. A satisfactory quantitative comparison of the zonal angular momentum computed from wind data with astronomical observations was made only relatively recently⁸ but it is now recognized⁹ that variations in length-of-day with frequencies between about 0.2 cycles yr⁻¹ and 4 cycles yr⁻¹ are mainly a consequence of an exchange of angular momentum between the Earth and the atmosphere. This conclusion was substantiated by Lambeck and Hopgood in a recent compilation of ten years of zonal angular momentum¹⁰. At higher frequencies (i.e. ≥ 4 cycles yr⁻¹) the atmosphere may contribute significantly to the length-of-day spectrum^{1,9}. A quantitative comparison of the length-of-day observations with the zonal winds in this frequency range has now been attempted by Hide and co-workers, (this issue of *Nature* p141) based on data collected in 1979 during the first CARP Global Experiment.

Hide *et al.* have attempted to evaluate the zonal component of the angular momentum of the atmosphere at 12 hourly intervals using wind and pressure data collected during two 8 week periods in 1979. Where observations were incomplete they were supplemented by values deduced from numerical circulation models. A comparison with the astronomical length-of-day data, particularly for the second period, indicates a generally good agree-

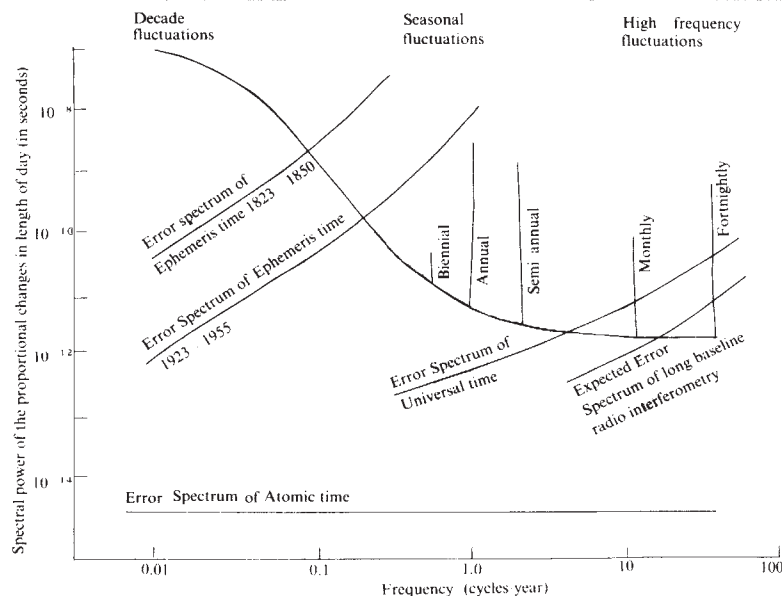


Fig. 2 Schematic spectrum of the proportional changes in length-of-day based on astronomical observations taken since the early nineteenth century. The error spectrum of Universal time is that which can be attained with astronomical observations since the introduction of atomic time. The error spectrum of ephemeris time is appropriate to the older data for the indicated time-intervals.

ment and confirms that the atmosphere makes a significant contribution to the high frequency changes in length-of-day. In particular, observed changes in length-of-day over five day periods can be attributed to rapid exchanges of angular momentum between the solid Earth and the atmosphere. From Hide *et al.*'s Fig. 3 it is also clear that shorter period, atmospherically induced, changes in rotation can be anticipated when the new methods of observing the rotation are fully exploited. What Hide *et al.*'s results show is that for precise and high-resolution length-of-day observations to be of geophysical use, the zonal wind field must be globally known with an accuracy and resolution that is at least as good as that collected during the first CARP Global Experiment. Such a major undertaking is justifiable for meteorological reasons. If future work substantiates Hide *et al.*'s conclusion then it may also become possible to use the high frequency and irregular length-of-day fluctuations as global constraints in numerical modelling of the circulation

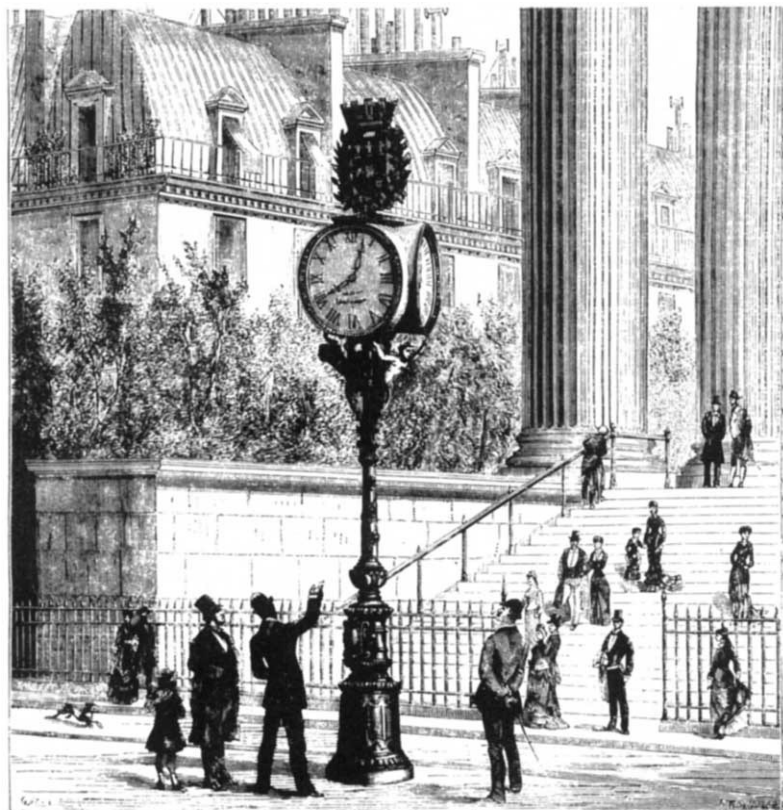
and, in particular, this may lead to a better understanding of how the atmospheric torques are distributed over the Earth's surface.

Towards the other end of the length-of-day spectrum, meteorological influences do not appear to be very important^{1,10} although some contribution cannot be excluded. Recently it has been suggested by Currie¹² that there is an 11 year length-of-day fluctuation associated with the solar cycle. Such a suggestion has been made previously¹³ but this conclusion is hard to reconcile with the quality of the earlier astronomical data. For example, prior to 1850 the astronomical data becomes only barely significant when averages are taken over 10 years while from 1923-55 only 5 year averages are significant (Fig. 2). Only since the introduction of atomic time in 1955 can one be certain that fluctuations with about 10 year periods will reflect real changes in rotation. This being said, a relation between solar activity and the Earth's rotation cannot be dismissed if there is a solar influence on the zonal circulation as was suggested most recently by Nastrom and Belmont¹⁴. Attempts at evaluating the contribution of the atmosphere to long period length-of-day variations have not been wholly successful through lack of complete and reliable meteorological data but they do indicate that the winds can contribute at most about 10-20% to the decade length-of-day fluctuations^{1,10}.

Partly by an elimination process, core-mantle coupling processes^{1,15} remain the most likely contenders for these decade fluctuations but here, as at the higher frequencies, the atmospheric contributions should be eliminated before other geophysical mechanisms can be reliably evaluated. Hide *et al.*'s results shows that this indeed can be done. □

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The Pneumatic Clock on the Place de la Madeleine.

100 years ago

To distribute the time with accuracy and uniformity in a large city is a problem of great utility and extreme importance. This problem has been all but completely solved by the

pneumatic clocks erected since March last in the principal streets of Paris and among a considerable number of subscribers, who, for a halfpenny a day, receive dials with pneumatic receivers established in the public streets and in private buildings.

From *Nature* 22, 8 July, 126, 1880.

Neurological mutants affecting myelination

from Richard P. Bunge

THEIR names (twitcher, quaking, jimpy, shiverer, trembler and dystrophic) suggest characters from a Dickens novel. They are, in fact, colloquial designations of the neurological abnormalities characterizing 6 strains of mutant mice, each with severe abnormalities of the myelin sheath within some part of their nervous system. These mutants provide important models of human disease and were the subject of a recent international meeting.*

The spiral membrane of the myelin sheath is formed by an intriguing feat of cellular acrobatics. The myelin forming cell extends a cellular process over a length of nerve fiber and deposits an expanding sheet of plasma membrane as a 'jelly roll' around the axon. As this cell process extends, the cytoplasm between the layers of plasmalemma is extruded and the spiralled membrane compacts into a multilayered

sleeve; this provides the insulating properties that increases conduction velocity. The myelination process, which occurs during the fetal and neonatal period, requires a large metabolic investment in the synthesis of prodigious amounts of new cell membrane; about 1/3 of the weight of the mature mammalian central nervous system is myelin.

Each of the curiously named myelin deficient mutants appeared on stage at the Seillac meeting but for one it was a debut. Twitcher provides a direct example of the results of a degradative enzyme deficiency in myelin-forming cells. This autosomal recessive mutant, first observed in 1976 at the Jackson Laboratory, has been studied by Duchon and his colleagues at the Institute of Neurology in London. They have observed that both the central nervous system (CNS) and peripheral nervous system (PNS) contain excessive numbers of macrophages laden with peculiar crystalloid inclusions and multiangular bodies. These were found in areas of myelin loss and closely resembled

cells that occur in the human degenerative disorder known as human globoid cell leukodystrophy, or Krabbe's disease. When Suzuki and Kobayashi at Albert Einstein in New York measured enzyme activities in this mutant they found a severe shortage of the enzyme that catalyzes the breakdown of one of the most prominent myelin glycolipids, cerebroside, to ceramide and galactose. Whereas myelination begins in a relatively normal manner in these mice, the amount of myelin present progressively declines as cerebroside accumulates. The twitcher mouse thus seems to provide a model of the fatal Krabbe's disease.

Such exact modelling of human disease is uncommon but two of the other mouse mutants present pathological pictures resembling rare hereditary human disease. In the X-linked mutant jimpy (*msd* seems to be an allele) there is little myelin and the few myelin forming cells present in the CNS appear immature. Because there is little evidence of myelin breakdown or lipid storage it is considered a primary failure of CNS myelination, a condition comparable to the Pelizaeus-Merzbacher disease in man. Infants often succumb during the first 3 or 4 years of life. The primary lesion in both the human and mouse disease is not known but work presented by Braun and co-workers of Montreal suggests that a membrane precursor to myelin accumulates in the jimpy mutant but is not converted into myelin.

The mutant trembler presents primarily PNS abnormalities including abnormal and sparse myelination with the concurrent deposition of connective tissue components which in time enlarge the peripheral nerve trunks. Aguayo and colleagues of Montreal (*Soc. Neurosci. Symp.* 4, 362; 1979) note that conduction velocity is much slowed in these nerves, that axon diameter is reduced and that Schwann cell proliferation continues at abnormally high rates into adulthood. Drawing on their experience with nerve transplantation (including the transplantation of human nerve biopsy specimens into immunosuppressed mice), this group has shown that trembler Schwann cells express their characteristic abnormality with axons in normal mice, and that Schwann cells from patients with Charcot-Marie-Tooth disease (an hereditary human disease with marked peripheral nerve hypertrophy) express a very similar phenotype with normal mouse axons.

The dystrophic mouse was discovered in 1975 (Bradley & Jenkinson *J. Neurol. Sci.* 25, 249; 1975) and was initially considered a model of muscle disease. It has been found to have a peculiar lesion in the nerve roots of its PNS, involving a more or less total failure of Schwann cell

*A meeting on Neurological Mutations affecting Myelination was held at Seillac, France, April 13-17, 1980. The organizer was Nicole Baumann and support provided by the French agency, INSERM. The proceedings will be published by Elsevier — North Holland.

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performance in these areas. Bunge and colleagues from St. Louis presented arguments at the meeting that this type of lesion could best be explained by an abnormality of extracellular matrix (ECM) within the nerve, i.e. a deficiency in the microenvironment of the Schwann cell. This contention derives from evidence that Schwann cell development is retarded if only axonal contact is available without simultaneous contact with appropriate ECM material (Bunge & Bunge *J. Cell Biol.* **78**, 943; 1978).

Centre stage was reserved for a relatively new mutant, shiverer (the mutant *mld* may be an allele). Interest in this mutant derives from the specific lack of one of the major myelin components — basic protein (Dupouey *et al. Neurosci. Letters* **12**, 113; 1979). This constituent is of special interest

for it causes a severe and sometimes fatal immune response when presented as an antigen with Freund's adjuvant. It previously had been assigned a quite specific role in normal myelin structure — that of stabilizing the compacted membranes of the myelin sheath. It was therefore not unexpected that CNS myelination was found to be severely deficient in this mutant (Privat *et al. Neurosci. Letters* **12**, 107; 1979) but it was surprising that myelin within the PNS, which also lacks basic protein, was quite normal in appearance, even at the level of the electron microscope (Kirschner & Ganser *Nature* **283**, 207; 1980). This mutant was much discussed at the Seillac meeting; several attendees suggested that available data indicate a different role for basic protein in CNS and PNS myelin. □

Timing in embryological development

from Michael H.L. Snow & Patrick P.L. Tam

NORMAL tables of embryonic development give the impression that the developmental processes are neatly co-ordinated, both spatially and temporally. The precision of timing of events is much stressed and frequently quoted in hours and minutes elapsed since the initiation of development. Certainly the synchrony observed between embryos of one species growing in a defined environment seems much more than mere coincidence. Evidence is accumulating that suggests a timing mechanism operates to control the emergence of developmental events. It is envisaged as a clock consisting of five components; the driving force (main-spring), operating an oscillatory mechanism (pendulum, balance wheel), which produces periodicity of emergent events (chimes on the hour), and all depending upon a linkage mechanism between oscillator and event (cog-wheels), and a capacity for adjustment in response to disturbance (correction for fast or slow running), (Brady *Biological Clocks*, Arnold, 1979).

In biological terms little is known about the driving force, the linkages or the homeostatic mechanisms but they can be presumed to exist. Studies have focussed on oscillators and the periodic events. More often than not the oscillatory mechanism has been ascribed to events in the cell cycle, DNA replication, cytokinesis. The premise that these reflect the operation of a clock has arisen from the characteristic regularity of the process in different tissues, and cell types. However the distinction made between proliferative

cycles, which merely increase the number of identical cells, and quantal cell cycles, which generate daughters with developmental options different from the mother cell (Holtzer *et al. Q. Rev. Biophys.* **8**, 523; 1975) suggests that differentiative events may be gated by a periodicity not necessarily associated with cell cycles. Recent work throws some light on this subject.

The cell nucleus is generally regarded as the control tower for cellular functions and it has been implicated in the timing of various developmental events. In Ascidian embryos the specific expression of the enzyme acetylcholinesterase in presumptive muscle cells of the neurula (Whittaker *J. Embryol. exp. Morph.* **55**, 43; 1980) has been shown to be under a stringent control apparently directly related to the number of DNA replications undergone in the nucleus. The timing of its appearance is unaffected when cell or nuclear divisions are prevented by metabolic inhibitors (Sato *J. Embryol. exp. Morph.* **54**, 131; 1979). In the mouse embryo, cavitation of the morula to form a blastocyst seems more plausibly related to nuclear events since it is not governed by chronological time, nor interfered with by manipulation of cell number or suppression of cytokinesis (Smith & McLaren *J. Embryol. exp. Morph.* **41**, 79; 1977).

Nevertheless, the nucleus can be excluded from other closely timed developmental events. In the mouse the time of compaction of the morula prior to blastocyst formation is not altered by changes in cell number, cell division, nuclear: cytoplasmic ratio or inhibition of DNA replication (Johnson, Pratt & Handyside in *Cellular and Molecular*

Aspects of Implantation, Plenum, 1980). Similarly in *Xenopus* eggs periodic changes in contractile activity in the cortex of the cell are observed in perfect phase with cleavage divisions but persist in the absence of actual cell division and also continue when the cell is deprived of its nucleus (Hara, Tydeman & Kirschner *Proc. natn. Acad. Sci. U.S.A.* **77**, 462; 1980). Cytoplasmic or membrane associated mechanisms need to be considered for the control of these events. At the molecular level in the mouse the timing of synthesis of 'tissue-specific' polypeptides is the same in normal or experimentally disturbed embryos (Johnson, Handyside & Braude in *Development in Mammals* **2**, (ed. Von Blerkom & Brockway) North-Holland 1977; *Devel. Biol.* **44**, 148; 1975).

It is an attractive idea that the future development of the embryo is timed by a clock set in motion at the very beginning of development but is this feasible? The control of somitogenesis in Amphibian embryos is a later event that is precisely timed, regular and is currently explained in terms of a clock model (Cooke *3rd. Symp. Brit. Soc. Devl. Biol.* Cambridge University Press, 1977; Pearson & Elsdale *J. Embryol. exp. Morph.* **51**, 27; 1979; Elsdale & Pearson *J. Embryol. exp. Morph.* **53**, 245; 1979). In mammals the later molecular events concerning the *in vitro* appearance of amylase activity in pancreatic primordia (Wessels & Cohen *Devel. Biol.* **15**, 237; 1977; Spooner, Cohen & Faubion *Devel. Biol.* **61**, 119; 1977) and the onset of responsiveness to androgen by mouse mammary tissue (Kratohvil *Devel. Biol.* **61**, 358; 1977) seem to relate to chronological age rather than cell cycle parameters since the biochemical changes coincide with the timing expected *in vivo* despite the fact that tissue mass and cell number may be quite different from that in the embryo *in utero*.

It is unlikely however that the whole embryo is controlled by a single clock. Recent studies in the mouse in which embryos recover from a severe size reduction show that the neural ectoderm, the somatic tissues of somite and limb-bud, and the germ cell population recover according to different timetables and appear to be under separate intrinsic controls (Snow & Tam *Nature* **279**, 555; 1979). So, either the clocks can be changed independently at tissue or organ level by an exogenous insult or, during development when various cell populations are set aside each one becomes a mosaic of time zones. It is intriguing to speculate whether the obvious co-ordination of tissue interactions in normal embryogenesis arises as the result of the harmonics generated by signals of differing periodicity originating from the different time zones. Such a system is reminiscent of the phase-shift model for spatial and temporal organisation proposed by Goodwin and Cohen (*J. Theoret. Biol.* **25**, 49; 1979). □

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Cree-Ojibwa hunting and the hare-lynx cycle

from Robert M. May

MANY animal species exhibit well defined cyclic variations in abundance. Of particular fascination to ecologists are the dramatic cycles shown by many mammals in the boreal region of North America; these include the 3–4 year cycles of mice, voles and lemmings and the 10–11 year cycles of the snowshoe hare, lynx and other fur bearers. Indeed the hare-lynx cycle, which shows pronounced and regular oscillations extending back nearly 200 years, is presented in essentially all introductory ecology texts as the classic example of prey-predator oscillations (with discussion centering on whether the driving mechanism is the predation of lynx on hare, or predation of hare on their food supply with the lynx then entrained). A most readable review is by Hutchinson¹.

The cycles in populations of fur bearing animals are, in the main, deduced from records of pelt sales, kept by the Hudson's Bay Trading Company and administrative agencies of the Canadian North. From time to time, people have questioned the assumption that these records accurately reflect the abundance of the harvested species. Thus Elton and Nicholson² and Keith³ have considered — and dismissed — the possibility that fluctuations in prices, caused either by business cycles in nineteenth century Europe or by factors intrinsic to the fur trade, have driven the cycle. Cyclic epidemics of disease affecting the trappers have also been suggested, but seem unlikely^{4,5}. A more substantial suggestion, made independently by Gilpin⁶ and Weinstein⁷, is that pelt records may reflect changes in the foraging strategy of the trappers, rather than changes in the population densities of the fur bearing animals.

Winterhalder⁸ has recently combined ecological studies with historical evidence about the foraging practices of Cree and Ojibwa Indians in northern Ontario, in an exploration of the extent to which apparent population cycles are artefacts of fur trapping practices.

Winterhalder first surveys studies showing that hare was a major component in the diets of boreal forest people. Particularly in the nineteenth and early twentieth centuries, when moose were scarce in, or absent from, northern Ontario (and possibly also from parts of Manitoba and Quebec), hare and fish were the main items in native diets. Winterhalder next examines the energy budgets for foraging for hare by Cree. In years of peak abundance there are around 1,200 hare per km², and he estimates a net acquisition rate of around 5,200 kcal per hr for time invested in snaring hares. At the trough of the hare cycle, the creatures are patchily

distributed, but average around 13 hare per km² and their net yield to Cree hunters is about 1,200 kcal per hr. (These figures are based on trapping with stationary wire snares, which were not widely used before 1910; the older balance poles with snare loops made of string were probably not more than 10% less efficient). These figures suggest hare snaring would allow trappers to feed their families with relatively small investments of time and energy, when hare were abundant; the activity consumed significantly more time and energy when hare were relatively scarce. Finally, Winterhalder shows that⁸ "until the twentieth century interior fur trappers could not exchange pelts for significant quantities of food. Dry goods, metal implements, tea, tobacco, and in some cases alcohol were available, but it was a recurrent problem for trading companies to transport or obtain sufficient food provisions even to maintain trading post personnel." Thus native trappers seldom had the option of exchanging pelts for food, and in times of shortage the pursuit of species highly ranked as food-producing resources necessarily had priority over those valuable mainly for their pelts.

These facts and inferences can be recast in the language of MacArthur and Pianka's⁹ theory of optimal foraging. To this end, Winterhalder separates the species sought mainly for food from those sought mainly for pelts, making allowance for some intermediate types. The food species, ranked roughly in the order of their net value (food value less costs of pursuit and capture), include moose, hare, beaver, fish and muskrat. The fur bearers include lynx, otter, mink, marten, fisher, beaver, muskrat, and hare. When highly ranked food resources (moose and hare) were plentiful they provided ample food that could be easily obtained (often by women and adolescents), allowing male trappers to⁸ "concentrate on the second economy represented by the fur trade". But when hare became scarce (and with moose rare or absent throughout much of the nineteenth century), more attention had to be devoted to lower ranking food resources, such as fish and muskrats, thus lowering the overall efficiency of the food quest. This in turn restricted the time available for participating in the fur trade; nor could furs be exchanged for staple foods. As a result, pelt-counts should be expected to cycle in parallel with the population abundance of hare.

Winterhalder also quotes from trader's reports, compiled by Bishop¹⁰ from the Hudson's Bay Trading Company Archives. For instance, "the Indians say that they cannot hunt furs for want of Rabbits for they say that there is a few Martens on thir Lands but they cannot

Make Traps for want of Provisions to live on."

Several interesting predictions follow from this analysis.

First, the above mechanism would have the peak abundance of pelts of most fur bearers, such as lynx, fisher and marten, occurring roughly contemporaneously with the peak of hare abundance (and therefore, presumably, the peak of hare pelt-counts). The dynamical properties of prey-predator associations, on the other hand, would have the predator populations lagging those of their hare prey, typically by about one quarter of the period of the cycle. This dichotomy is not discussed by Winterhalder, who observes that either explanation of the cyclic fluctuations in those animals sought mainly for fur (and not for food value) is roughly consistent with the data. Gilpin's⁶ analysis, however, suggests the predator cycles do not lag significantly behind the hare cycles; this work could be taken to support the view that cycles in pelt records of fur bearers are artefacts of Cree-Ojibwa foraging strategies, rather than measures of the population dynamics of the animals themselves.

Second, Winterhalder observes that pelt-count cycles are more pronounced in the nineteenth than in the eighteenth and twentieth centuries. This was also a time when moose and caribou were relatively absent from the hunting grounds of the Cree and Ojibwa; without this important food source to buffer fluctuations in the hare population⁸, "the correlation between hare abundance and native attention to the fur trade should be strongest".

Third, muskrat is an interesting species in that it ranks relatively low both as a food source and in fur value. Winterhalder suggests that in times of hare-feast muskrat would have been spurned, but that in times of hare-famine the native hunters drew heavily on this less desirable food source, and sold the ensuing pelts. The resulting prediction is that peaks in muskrat pelt-counts should come in the troughs of hare abundance, and have nothing to do with the absolute abundance of muskrats as such. A somewhat irregular pattern of this kind is actually observed, particularly in the second half of the nineteenth century (when, as noted above, all patterns were more marked, by virtue of the absence of

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moose).

There are, of course, extensive field studies of the population dynamics of snowshoe hare, and, to a somewhat lesser extent, of lynx. These studies show the populations do in fact cycle, with periods of 10–11 years. Indeed, Bryant (of the University of Alaska) has shown that many of the deciduous plants browsed by snowshoe hare in Alaska manifest defensive responses to heavy browsing, by producing resins or other compounds that are repellent or toxic to hares¹¹. The time lag in mounting this response is around 2–3 years; such a time lag in the regulatory mechanism will tend to produce popula-

tion cycles of period $4 \times (2 \text{ to } 3) \approx 10$ years in the overall prey (food)-predator (hare) system¹². Species predatory upon the hare will then track this cycle.

In short, there is direct evidence to show that populations of hares and other boreal fur bearers do exhibit oscillations. But the nature of the foraging economy of the Cree-Ojibwa hunters must be kept in mind when we try to draw inferences about populations from records of pelts traded; pelt counts tend to amplify and sharpen the underlying population cycles of fur bearers (such as fisher and marten) and some cases (such as the out-of-phase muskrat cycles) may be pure artefacts. □

arose in an argument over central ocean productivity. The arguments for order-of-magnitude greater production would require unrealistically high differences in quantum efficiencies between coastal and open-ocean regimes. Production would be 1–2 grams carbon $\text{m}^{-2} \text{ day}^{-1}$ in both yet the fraction of sunlight absorbed by phytoplankton is an order-of-magnitude greater in coastal than oceanic waters.

Many of those present were interested in photosynthesis light curves (T. Platt, Bedford Institute, Dartmouth, Canada) the regulation of chlorophyll, and other photosynthetic pigments in algal cells (P. Falkowski, Brookhaven National Laboratory; J. Marra, Lamont-Doherty Geological Observatory) and the vertical distribution of photosynthetic pigments in the ocean (S. W. Jeffrey, CSIRO, Australia). Falkowski presented the interesting hypothesis that regulation of cellular chlorophyll content by light may involve a competition for ATP between ammonium assimilation leading to protein synthesis on the one hand and the use of glutamate in the biochemical synthesis of chlorophyll on the other. Both Marra and Falkowski suspect that the rapid adjustment of cellular pigment content with changing irradiance and light quality (i.e. with depth) can serve as tools to study rates of vertical water motions in the photic zones. Here biology may provide information on the near surface circulation that is difficult to assess with physical observations.

Developing conceptually adequate mathematical models of ocean photosynthesis and phytoplankton growth rate (related to photosynthetic production by the phytoplankton standing stock) has been a difficult task. Nevertheless, T. Bannister (University of Rochester) has achieved a satisfying model which could be fitted to the old data of J. Myers and J. R. Graham on *Chlorella* and the new data of E. Laws (University of Hawaii) for the diatom *Thalassiosira fluviatilis*. The latter data set and modelling includes nitrogen, phosphorus and light-limited growth. Several generalizations from the work will be useful in the field assessments: both the dark respiration rate and the ratio of chlorophyll α to carbon in phytoplankton are linear functions of growth rate, and the photosynthesis light saturation term is not altered when growth rate is reduced by nutrient limitation.

Titbits of information were offered in profusion in the lecture hall and in the corridors. For example, R. Rivkin (Johns Hopkins University) has succeeded in measuring with ^{14}C the photosynthesis of individual cells of some large dinoflagellates. Cell division in diatoms seems to be less under the control of a biological clock than in dinoflagellates (S. W. Chisholm, MIT). It is still not clear whether photo-

Primary productivity in the sea

from Richard W. Eppley

PEOPLE have been measuring primary production in the oceans using the ^{14}C method of E. Steemann Nielsen for nearly 30 years, yet they still argue about the accuracy and meaning of the measurements. Some of the debate concerns methodological details of little interest beyond the circle of measurers, but a larger part questions our understanding of photosynthetic mechanisms, the absorption of sunlight by plants in the sea, quantum efficiency of photosynthesis as a function of depth and submarine irradiance, metabolic adaptations of algae and other matters of broader interest. Oceanographers debated these issues 2–5 June at the 31st Brookhaven Symposium on Biology in the presence of experts in the laboratory study of photosynthesis and algal physiology. The productivity of coral reefs and seaweeds was also discussed along with the significance of global oceanic production for human affairs.

Measurement of ocean photosynthesis is no longer the exclusive territory of algal ecologists and physiologists as the experimental seawater samples contain minute animals that graze on the contained phytoplankton and bacteria — a microcosm exists within the productivity bottles. While this has long been known, we now realise that we must assess the flow of photosynthetic products among tiny animals and bacteria — the province of microbiologists and protozoologists — as well as among phytoplankton in order to estimate primary production. There is also concern that many oceanic species may not survive the mechanical and chemical insults of the measurement conditions and thus photosynthetic production may be underestimated by present methods. The size of the underestimate is unknown. There is agreement, however, that the problem is more extreme for the central oceanic gyres, such as the Sargasso Sea and

comparable regions in the South Atlantic and North and South Pacific. Guesses of the magnitude of the underestimate seem to range from a factor of two to an order of magnitude for these areas. However most of a day's production in such regions is eaten, digested, defecated, decomposed and the organic-bound nutrients recycled during the day-night cycle of the 24-hour day. Thus, even those measurements suggesting higher values for production, such as daytime increases in oxygen in the water and in the concentration of organic particles, living and dead, involve daytime increases that disappear by the following dawn. Upward revision of the photosynthetic rates would not imply more food for fish although it would require some new thinking about the rates of elemental recycling in the surface waters and the ecological significance of microbial food webs. Our understanding and the validity of 30 years of historical measurements of production in the central oceans are more at stake than practical matters.

Z. Dubinsky (University of Bar Ilan, Israel) confirmed J. E. Tyler's earlier finding that the quantum yield of photosynthesis is very low at the sea surface and increases to near maximum expected values at the bottom of the photic zone. Jack Myers (University of Texas, Austin) explained why the theoretical maximum quantum yield (0.125 from the Z-scheme of photosynthesis with equimolar reaction centres for photosystems I and II) is rarely attained: the photochemical reaction centres are not always open to traffic. A. Ley (Rockefeller University, New York) found the reaction centres of unicellular red algae were not present in equimolar amounts; this would reduce the maximum quantum yield expected in dim light, depending on the proportion of reaction centers I and II. The practical problem of the quantum efficiency of photosynthesis

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Interferon nomenclature

AT a recent, specially-convened meeting an international group of scientists attempted to devise a system to define and classify the interferons*. The following is the unanimous report from the group.

The committee accepted the definition for interferon: "To qualify as an interferon a factor must be a protein which exerts virus non-specific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and protein."

The preferred abbreviation for interferon is IFN, each interferon to be identified according to animal of origin: e.g. human (Hu IFN); murine (Mu IFN); bovine (Bov IFN); rat (Rat IFN); chicken, porcine, feline, equine interferon, etc. However, it may be necessary in some cases to apply generic nomenclature: e.g. monkey = *Rhesus* interferon, etc.; fish = *Salmo* interferon, etc.; bat = *Taderida* interferon etc.

Interferons will be classified into types on the basis of antigenic specificities, type designations to be alpha (α), beta (β) and gamma (γ), corresponding to previous designations of leukocyte (Le), fibroblast (F), and type II (immune) interferons, respectively. The old terminology of 'leukocyte', 'fibroblast' and 'immune' interferons were, by committee consensus, abolished, as they clearly were misnomers: both leukocyte and fibroblasts can produce each of these two types of interferon, and 'immune' interferons are often induced by mitogens rather than by immune recognition reactions. Also, the abbreviation 'F' has often been used to designate either fibroblast or fast migrating interferons. Alpha and beta interferons are usually acid-stable and correspond to what have

been called type I IFNs; gamma interferons are usually acid-labile and correspond to what has been called type II IFNs. If there are discovered to be classes of IFN which are not presently recognized, they could be designated sequentially IFN-delta, epsilon, etc. The Table lists the new nomenclature for human and murine interferons and the corresponding designations which have been or are being employed. Interferon preparations may contain more than one type: for example, interferons derived from human lymphoblastoid cells and interferons from murine fibroblasts contain both IFN- α and IFN- β . These can be designated as to predominant type. Thus, the interferons presently employed in clinical trials are either HuIFN- α or HuIFN- β , or admixtures thereof.

The committee's recommendations apply at present only to human and murine IFNs, since there is insufficient information about IFNs from other animal species to allow differentiation into types. Workers using IFNs from other species are encouraged either to attempt preliminary classification based on antigenic homology with existing antisera specific for human or mouse alpha, beta or gamma IFNs, or to establish sequence homology with the known types of human and mouse interferons.

It is recognized that there may be size, charge, sequence and other heterogeneities within the designated IFN types. Currently, there is insufficient information available about the nature of these differences to allow designation of specific

subtypes. Properly documented differences in molecular size appear to be useful parameters for differentiation until more stringent criteria (amino acid sequence, monoclonal antibodies) become available. Molecular weight designations may be indicated in parentheses, e.g. Hu IFN- α (18K) or Mu IFN- β (38K), or subtypes of IFNs based on specific amino acid sequence differences can be classified as Hu IFN- α_1 , HuIFN- β_2 , etc.

Indication of interferon origin for those that behave antigenically similarly may also be helpful. Thus, interferon from human lymphoblastoid cells and interferon from human leukocytes are both classified antigenically as HuIFN- α , yet these interferons differ in certain amino acids. Therefore, the nomenclature HuIFN- α (Ly) or HuIFN- α (Le) may be used until such time as specific differences in amino acid loci have been established and subtype designations can be assigned. Also, it is now recognized that there are a number of forms of HuIFN- α that differ in certain amino acids; eventually these may be designated according to specific amino acids and their locations. Schemes for such designation of subtypes of each of the major IFN types will be developed at subsequent meetings of the Nomenclature Committee. The next meeting of the committee will be held in April 1981. Suggestions and comments for consideration by the committee should be forwarded to the committee's Chairman.

The committee hopes that scientists and scientific journal editors will adopt the proposed nomenclature to avoid the proliferation of terms for the same substances and the indiscriminate naming of newly discovered factors.

Old and new nomenclature for human and mouse interferons

New Nomenclature	Old Nomenclature	
	Human	Mouse
IFN- α	Le (leukocyte), type I, pH 2 stable, foreign cell-induced	F (fast), C, type I, pH 2 stable
IFN- β	F (fibroblast), Fi, type I, pH 2 stable	S (slow), A,B, type I, pH 2 stable
IFN- γ	IIF (immune), type II, T, pH 2 labile, antigen-induced, mitogen-induced	immune (IIF), type II, pH2 labile, T, antigen-induced, mitogen-induced

The meeting was sponsored by the National Institute of Allergy and Infectious Diseases and the World Health Organization — US National Centre on Interferon and was held at the National Institute of Health in Bethesda, Maryland on March 7, 1980. Those attending were: William E. Stewart II (Chairman; Interferon Laboratories, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, US), J. Edwin Blalock (Galveston, TX), Derek C. Burke (Coventry), Charles Chany (Paris), June K. Dunnick (Bethesda), Ernesto Falcoff (Paris), Robert M. Friedman (Bethesda), George J. Galasso (Bethesda), Wolfgang K. Joklik (Durham, N.C.), Jan T. Vilcek (New York), Julius S. Youngner (Pittsburgh) and Kathryn C. Zoon (Bethesda).

respiration is significant for marine phytoplankton (J. Burris, Pennsylvania State University). On a global average the fate of about 80% of the primary production is to be recycled within the photic zone. The relative importance in nutrient recycling of bacteria, microzooplankton, larger zooplankton and nekton in the photic zone appears to vary between the continental shelf, the slope water and the open ocean (W. G. Harrison, Bedford Institute, Dartmouth, Canada).

Harrison's paper is indicative of the

expanding interest of biological oceanographers in the fate of primary production. Globally, about 20% of it appears to sink out of the photic zone. J. Walsh (Brookhaven National Laboratory) postulated that a large fraction of this sinking flux on the continental shelf off New York is buried in the sediments of the upper continental slope, as shown by the relatively high sediment organic carbon content and a low (~6) ratio of carbon to nitrogen in the organic matter. Models of carbon flows in the circulation of the deep ocean (M.

Fiadeiro, Yale University, New Haven) suggest what the magnitude of the sinking flux of organic carbon may be. Unhappily, the experimental measurements of the flux in the North Pacific using particle interceptor traps are inconsistent with the models. Further collaboration among the several oceanographic disciplines seems required to clarify such problems and especially to define the significance of sinking organic particles as sinks and transporters of atmospheric carbon dioxide from fossil fuel burning.

ARTICLES

Zeolitic structures as revealed by high-resolution electron microscopy

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Interpretable images at 3–5 Å resolution of synthetic A-type zeolite suggest possible mechanisms of vitrification and introduction of dislocations into zeolitic structures. Techniques may be developed to extend the lifetime of these, and possibly other, beam-sensitive cage or tunnel structures. Evidence also exists for $\text{Si}^{4+}/\text{Al}^{3+}$ ordering in the aluminosilicate framework.

ZEOLITE silicates are of considerable practical and academic interest in view of their role as catalysts, ion-exchangers, gas-purifiers, environmental cleaning agents and, following vitrification, as potentially useful matrices for the burial of radioactive waste¹. For both channel and cage varieties synthetic adaptation has given rise, *inter alia*, to ultrastable zeolites possessing specific catalytic activity for either the cracking of petroleum or the production of gasoline from methanol, using highly silaceous hydrophobic molecular sieve solids such as ZSM-5 (ref. 1). The general chemical formula of a zeolite is $\text{M}_x\text{D}_y\cdot\text{Al}_{x+2y}\text{Si}_{n-(x+2y)}\text{O}_{2n}\cdot m\text{H}_2\text{O}$ where M and D may be mono- and divalent cations respectively, usually alkalis and alkaline earths in nature, but other ions in synthetic varieties. Dehydration of zeolites is usually complete at 350 °C (refs 1, 2) and is often reversible: dehydrated zeolites are strong desiccants, however, and molecules other than water may rapidly fill the channels¹.

Crystallographic studies and their implications for practical applications have been reviewed elsewhere^{1, 2} and an atlas of known zeolite structures is available³, although most structural work has relied on X-ray methods². Gard⁴, however, used electron diffraction to obtain evidence for a pseudosymmetric cell doubling of a 'Union Carbide' zeolite A. Although his data led to a refinement of the structure when the weak reflections were measured by X-ray goniometry⁵, the wide range of zeolitic structural variations remains to be fully appreciated¹. Thus there may be marked differences between hydrated and dehydrated forms² and it is not always practicable to locate all of the M, D and H_2O sites or the sites of other ion-exchanged entities. Often crystals are too small ($\sim 0.1\ \mu\text{m}$) for X-ray studies. Moreover, there are apparently many possibilities for the occurrence of short-range order, antiphase domain structures, topological transformations, coherent intergrowth, polytypism and stacking disorder. These are particularly relevant in considering the synthesis of new phases having desirable catalytic, sieve, or other properties. In several areas of solid state chemistry, such as transition metal oxides and chalcogenides, alloys and silicates high resolution electron microscopy has proved invaluable in elucidating the local atomic arrangement which may, in many cases, be lost or not readily detected using X-ray diffraction alone^{6, 7}.

There have been very few reports of high-resolution studies of zeolites. Although Menter⁸ first observed images showing $d_{111} = 14.7\ \text{\AA}$ spacings in a synthetic faujasite in 1958 it was

not until 1978 that Sanders⁹ obtained the first high-resolution ($\sim 3\ \text{\AA}$) two-dimensional image of a (Linde L) zeolite. Sanders, however, used a zeolite as a resolution test object for a 1-MeV electron microscope and no new information about the structure was obtained. This sparsity of electron microscopic data stems principally from the electron beam sensitivity of zeolites, which vitrify within seconds, or at most minutes on examination in normal electron microscope illumination conditions. This beam sensitivity is probably linked with the water present in zeolites.

We report here the observation at high resolution (3–5 Å) of two-dimensional structure images of a synthetic Na-A zeolite. Interesting changes associated with the crystalline/glass transformation and the first observation of dislocations in zeolites are also described.

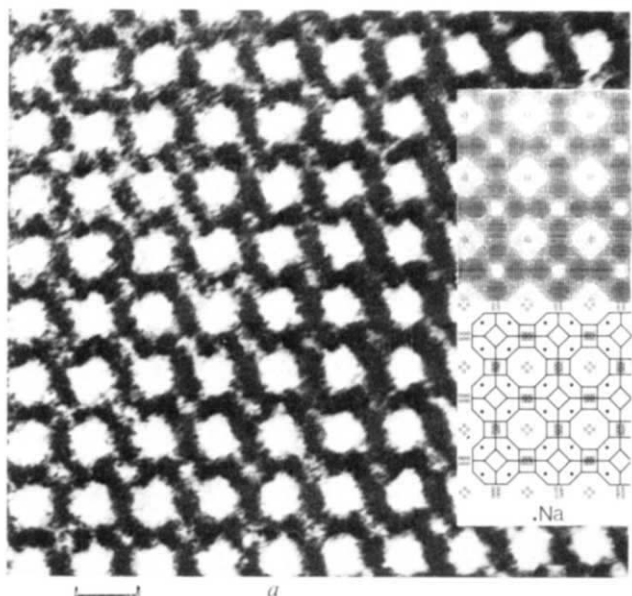


Fig. 1 *a* High-resolution image of zeolite A, [001] projection. *b*, Computer simulated image corresponding to $C_s = 2.5\ \text{mm}$; crystal thickness = 50 Å; objective lens defocus, $-1,040\ \text{\AA}$; effective resolution, 3.1 Å. *c*, [001] projection of zeolite A aluminosilicate framework, Na atoms are indicated by ● and ○. Scale bar, 12.3 Å.

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Stabilization of zeolites for electron microscopy

We hypothesize that the primary source of the crystalline/glass transformation induced by the electron beam is the production of ionized species, in particular hydroxyl ions, which lead to bond softening and bending in the aluminosilicate framework. This, when accompanied by elastic stresses associated with dehydration, will strongly favour collapse of the structure and consequent transformation to a grossly disordered (glassy) state. We, therefore, set out to dehydrate some well crystallized zeolites by heating in air, or *in vacuo*, before examination in the electron microscope, by ion-exchanging H_2O by inorganic salts, either during or after synthesis, and began a systematic study of a wide range of zeolites to discover which of these are least beam sensitive. However, in the case reported here, we were able to increase the longevity of the crystalline state significantly by simply pumping the specimens overnight *in situ* in the electron microscope (at $\sim 10^{-7}$ torr).

Experimental

The NaA zeolite samples used here were prepared by the Charnell method¹⁰. Their nominal stoichiometry is $\text{Na}_{12}(\text{AlO}_2)_{12}(\text{SiO}_2)_{12} \cdot 27\text{H}_2\text{O}$ when fully hydrated. Fine crystals, ~ 10 – $50\text{ }\mu\text{m}$ diameter, of this zeolite were placed in an agate mortar and crushed using a hard vertical thrust of the pestle, whereby a remarkably high incidence of $\langle 100 \rangle$ zone axis diffraction patterns were obtained. Such specimens were rapidly tilted into the symmetrical orientation using a JEOL-200 CX side-entry goniometer. Many such crystals showed good lattice images on the viewing screen and some of these persisted for a sufficiently long time (1–3 min) to enable objective lens astigmatism to be corrected and the optimum defocus established before photography. Exposure times were typically 4–11 s, using Kodak 4463 electron image film developed in D19 for 8 min.

Two sets of objective lens pole pieces were used having spherical aberration coefficients $C_s = 2.5\text{ mm}$ and 10 mm respectively. Calculations of the corresponding lens transfer functions at 200 kV showed that at the Scherzer defocus¹¹ the 'interpretable image' resolution limits were ~ 3 and $4.1\text{ }\text{\AA}$

respectively. Previously it had been shown that $3\text{ }\text{\AA}$ limit could be achieved, for example with parawollastonite and cordierite, only if a suitably reduced gun bias setting was used, along with low beam divergence (semi-cone angle $\alpha \leq 1\text{ mrad}$) and if all sources of acoustical vibrations were eliminated.

Experimental results

Figure 1a shows a high-resolution image for the $[001]$ orientation. Note the large white blobs and also much smaller white blobs linking the former along $\langle 100 \rangle$. The dark intensity surrounding the small tunnels can be just resolved into four dark blobs. Figure 2a shows a wider view of the crystal. A strip of essentially structureless contrast may be seen along the thin edge. This is presumably amorphous as it shows a characteristic lack of fringe contrast which extends progressively into the crystal with increasing exposure to the electron beam. At the same time the electron diffraction spots gradually fade giving way to a diffuse halo pattern. In many cases it was possible to record only two images before all structural detail was lost. The interface between glass and crystal is surprisingly sharp in Fig. 2a, showing unit cell steps ($\sim 12\text{ }\text{\AA}$ high) along $\langle 100 \rangle$ directions. Note changes in contrast giving a patchy appearance. Some patches (~ 150 – $200\text{ }\text{\AA}$ diameter) show a loss of fringe detail in one of the $\langle 100 \rangle$ directions (A), others show generally reduced (B) or virtually no (C) visible structure. Note especially that small islands of fringes (D) may be found, apparently completely surrounded by glass. These remnants of structure show fringes only along $\langle 110 \rangle$. Further inspection of Fig. 2a, by sighting along $[100]$, reveals what seems to be an extended dislocation (circled). Further dislocations are more obvious in Fig. 2b, which shows nearly the same area, but was recorded using larger objective lens defocus. Patches are now more clearly outlined, by dark lines resembling Fresnel fringes, and terminating half-planes (dislocations) are seen to be associated with the interface between patches of fringes misoriented by ~ 5 – 10° with respect to the bulk.

Figure 3 shows a pincer-shaped intrusion of glassy material into a NaA crystal. The latter introduces a dislocation and extensive (~ 100 – $200\text{ }\text{\AA}$) strained regions around the intrusion tip.

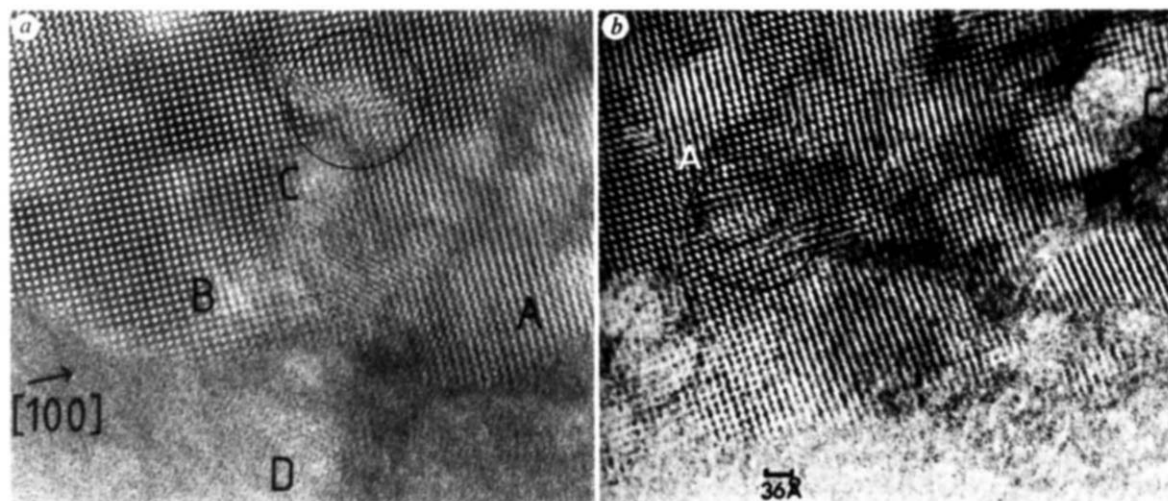


Fig. 2 a, b, Wider view of zeolite A crystal for two defects of focus showing development of glassy strip along thin edge of crystal and the appearance of patches $\sim 150\text{ }\text{\AA}$ diameter showing preferential loss of one set of $\{100\}$ fringes (A), weak (B) or absent structural detail (C) and islands of weak $\{110\}$ fringes (D) completely surrounded by glassy material. Note occurrence of extended dislocations (circled), sight along $\langle 100 \rangle$.

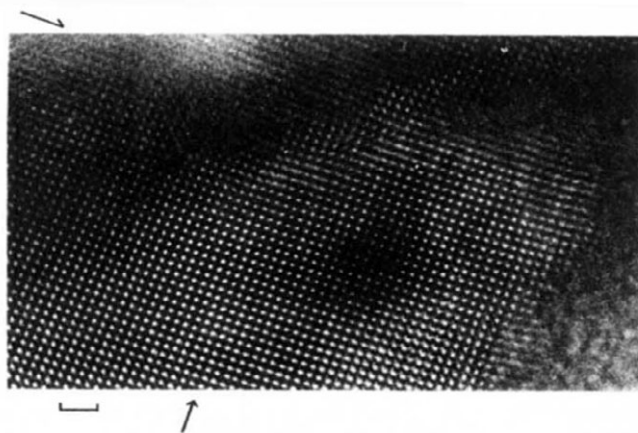


Fig. 3 Area of NaA showing pincer-shaped intrusion resembling a glass-filled Griffith crack. A dislocation may be seen by sighting along the rows of tunnels (indicated by arrows).

Discussion

The ideal chemical formula is $\text{Na}_{12}(\text{AlO}_2)_{12}(\text{SiO}_2)_{12} \cdot 27\text{H}_2\text{O}$ when fully hydrated, with cubic unit cell $a = 24.6 \text{ \AA}$, containing eight formula units⁵. The aluminosilicate framework contains two types of polyhedra: one a simple cubic arrangement of eight tetrahedra and the other a truncated octahedron of 24 tetrahedra (β cage). Their combination (Fig. 1c) produces a large cavity (α cage) with tetrahedral centres occupying the apices of a truncated cuboctahedron, free diameter $11 \text{ \AA}$. We have used the $12.3 \text{ \AA}$ pseudo-cell¹², space group $\text{Pm}\bar{3}\text{m}$, for convenience here, as there are not observable effects in the $[001]$ projection due to Si/Al ordering. However, we have obtained evidence, from electron diffraction patterns of $[110]$, $[11\bar{2}]$ and $[21\bar{3}]$ zones that Si/Al ordering does take place in our zeolite preparation. Thus hkl (all odd) reflections satisfying $h+l=2n$ were observed¹³. This confirms the recent criticism of space group $\text{Fm}\bar{3}\text{c}$ for NaA on the basis of high-resolution magic-angle spinning ^{29}Si NMR studies^{13,14}. The proposed Si/Al ordering scheme contradicts Loewenstein's rule¹⁵. Zeolite A is thought to retain its structure on dehydration, which is reversible, after which the cell parameter decreases by only $0.02 \text{ \AA}$ (ref. 2).

Computer simulated images were obtained using multislice techniques¹⁶ and programs previously developed by Jefferson *et al.*¹⁷. Computed images of zeolite NaA showed a faint grey blob in the centre of the α cage (large tunnels) if atomic coordinates for the Na atoms were included (Fig. 1b). These did not occur on the experimental images, suggesting that some Na atoms were either absent or had moved away from the centres of the tunnels on dehydration. In fact, the image match was improved by leaving out those Na^+ atoms having low partial site occupancy (indicated by open circles in Fig. 1c). Otherwise the image match was achieved assuming effective resolution of $3.1 \text{ \AA}$. Further improvements in resolution (to $2.4 \text{ \AA}$) are expected when ultra-high-resolution pole pieces ($C_s = 1.2 \text{ mm}$) become available. However, we have clearly demonstrated that high-resolution images of zeolitic silicates may be obtained and that details of the contrast may be matched by computer simulation. Both large and small channels have been located. Further calculations and even higher resolution experimental images are necessary to determine whether specific cation-exchange sites may be located, but we expect that certain heavy ions may be found. We have already shown that the images are sensitive, in

optimum defocus conditions and for sufficiently thin crystals, to the cation content of the larger tunnels.

NaA zeolite shows interesting contrast effects associated with transformation to the vitreous state. At least three processes seem to be operating. Thus, for Fig. 2a there is an extremely sharp interface, containing unit steps of $12 \text{ \AA}$, which seems to advance into the crystal one unit cell at a time, starting at the thin edge. Second, there are patches or islands of structure, B and C in Fig. 2a, which are misoriented by several degrees with respect to the bulk of the crystal. These sometimes show a deterioration in image visibility suggesting loss of crystallinity (A, B) whereas other patches appear completely vitreous. Patches of types A and B imply a preferential breakdown of the structure on $\{100\}$ planes whereas remnants of $\{110\}$ planes may continue to exist even in areas completely surrounded by glass (D). Apparently, vitreous regions $\sim 150 \text{ \AA}$ diameter form inside the bulk crystal, although we cannot rule out the possibility that regions having roughly circular cross-section are propagating into the crystal from upper or lower surfaces. Lattice rotation and the consequent production of dislocations may arise from the changes in cell parameter associated with the breakdown of $\{100\}$ planes or with elastic strain due to changes in density of partially or completely transformed material. However, inspection of the structural drawing (Fig. 1c) immediately suggests that the origin of preferential breakdown on $\{100\}$ planes is due to shear across the relatively weakly bonded structural units linking β -cages. This may be associated with the loss of Na and consequent rearrangement of the aluminosilicate framework, possibly by face-sharing of β -cages forming sodalite type structure units^{2,3}. (Note that sodalite may be obtained from zeolite A by a three-dimensional crystallographic shear operation $\{100\}\langle\frac{1}{2}100\rangle$). The circled extended dislocations in Fig. 2b,c may well represent the initial stages of this process. More detailed analysis of the defects shown in Fig. 2, may lead to structural models for the dislocations and for transition structures.

A third transformation mechanism is suggested by the dislocation and strained region, surrounding the pincer-shaped glassy intrusion in Fig. 3. This resembles a glass-filled Griffith crack. Thus eventually relatively large areas ($0.1\text{--}0.5 \mu\text{m}$) will be split off from the bulk crystal. This mechanism also leads to islands of crystalline or paracrystalline regions surrounded by glass. (Similar intrusions were observed in a $[011]$ zone axis image of zeolite A.)

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Atmospheric angular momentum fluctuations and changes in the length of the day

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Fluctuations in the angular momentum of the Earth's atmosphere correspond fairly well with equal and opposite fluctuations in the angular momentum of the Earth's 'solid' mantle. A decrease of 20% of the relative angular momentum of the atmosphere during the second half of May 1979 was accompanied by a speeding up of the rotation of the mantle causing the length of the day (l.o.d.) to decrease by 0.6×10^{-3} s. Although motions in the Earth's liquid core produce the more pronounced 'decade' variations in the l.o.d. there is no evidence that core motions produce l.o.d. variations on much shorter time-scales.

FLUCTUATIONS in the angular momentum $\mathbf{M}_a$ of the Earth's atmosphere are of considerable interest to geophysicists and astronomers concerned with the dynamics of the 'solid' Earth as well as to meteorologists concerned with the general circulation of the atmosphere and numerical models¹⁻⁶. All components of $\mathbf{M}_a$ exhibit fluctuations due to changes in the distribution of mass in the atmosphere and in the pattern of winds, and these will be associated with changes in the rate of rotation of the underlying planet and with movements of the poles. We are investigating these fluctuations as new wind and pressure observations become available from FGGE (First GARP global experiment where 'GARP' is Global Atmospheric Research Programme), which started at the beginning of December 1978 and lasted for 1 year. A detailed study of fluctuations in all components of angular momentum in the Earth-atmosphere system based on these new data will be reported elsewhere. Here we outline our determinations of the main contribution to fluctuations in the axial component of $\mathbf{M}_a$, namely that due to changes in the distribution and strength of zonal winds. (The main direct effect of redistribution of mass in the atmosphere is the alteration of the direction of $\mathbf{M}_a$ which is shown largely as the slight compensating movements of the pole of rotation of the solid Earth^{1,2}.) The results are a valuable check on established methods for measuring variations of the Earth's rotation on a time scale of several weeks, and should prove particularly useful in testing new methods for monitoring more rapid fluctuations of the Earth's rotation. These new methods⁴, notably very long base-line interferometry and laser ranging to artificial satellites and the Moon will eventually provide useful data on the general atmospheric circulation.

The density of the atmosphere decreases rapidly with height and more than 95% of the mass of the atmosphere is contained within the troposphere, which extends from the ground to an altitude of ~ 10 km, and the lower stratosphere. These regions of the atmosphere rotate faster on average than the underlying solid Earth with a typical relative speed of about 10 m s^{-1} . Exactly how this super-rotation (a term used for the generally more rapid relative motions found in the very tenuous regions at heights around 300 km) is produced and maintained against the tendency for friction to oppose differential motions between the atmosphere and the underlying planet is a central problem in dynamical meteorology.

In the absence of energy sources such as solar heating, internal radioactivity, and tidal forces due to the Moon, Sun

and planets, the solid parts of the Earth (crust, mantle and inner core) and fluid parts (atmosphere, oceans, liquid outer core) would rotate together as a rigid body with angular velocity Ω equal to the angular momentum $\mathbf{M} + \mathbf{M}_a$ of the whole system (magnitude $5.85 \times 10^{33} \text{ kg m}^2 \text{ s}^{-1}$) divided by its principal moment of inertia $I + I_a = 8.04 \times 10^{37} \text{ kg m}^2$ where I_a is the principal moment of inertia of the atmosphere. During the development of the three-dimensional and non-axisymmetric global atmospheric circulation following the imaginary switching-on of the main source of energy for atmospheric motions, solar heating, $\mathbf{M}_a$ would increase by an amount $\delta \mathbf{M}_a$ (magnitude $\div 1.5 \times 10^{26} \text{ kg m}^2 \text{ s}^{-1}$), associated mainly with the average super-rotation of the troposphere and lower stratosphere at average angular speed $|\delta \mathbf{M}_a|/I_a$ relative to the underlying planet, about the main rotation axis. As $I_a = 1.42 \times 10^{32} \text{ kg m}^2$ then $|\delta \mathbf{M}_a|/I_a \div 10^{-6} \text{ rad s}^{-1} \div 1.5 \times 10^{-2} |\Omega|$. To conserve the angular momentum of the whole system a change $\delta \mathbf{M} = -\delta \mathbf{M}_a$ in $\mathbf{M}$ would occur, largely manifested as a reduction in the magnitude of the angular velocity of the solid Earth by an amount $|\delta \Omega| \div |\delta \mathbf{M}_a|/I \div 2 \times 10^{-12} \text{ rad s}^{-1} \div 3 \times 10^{-8} |\Omega|$. Thus the l.o.d. $= 2\pi/|\Omega|$ would increase by about $3 \times 10^{-3} \text{ s}$ which is small in absolute terms but comparable in magnitude with the largest changes in the l.o.d. revealed by astronomical transit observations. Fluctuations in the relative angular momentum $(\mathbf{M}_a - I_a \Omega)$ of the atmosphere of 20% in magnitude could therefore produce changes in the length of the day of $6 \times 10^{-4} \text{ s}$ and as there are observed changes of this magnitude on meteorological time scales of weeks and months it is reasonable to suppose that these changes might be produced by the fluctuations in the net couple $\mathbf{C}$ applied by the atmosphere to the underlying planet¹⁻³.

The couple $\mathbf{C}$ is a result of the instantaneous imbalance between the positive couple exerted by the atmosphere on the Earth in mid-latitudes, where the zonal component of surface winds is generally eastward (westerly) and the negative couple in tropical regions, where the surface (Trade) winds have a westward (easterly) zonal component, and to a much lesser extent in polar regions, where westward surface flow is found. Although comparatively large (and negative) during the setting-up process, this imbalance usually amounts to no more than a few per cent and fluctuates in sign, exhibiting persistent quasi-biennial, seasonal and irregular variations. Direct determinations of $\mathbf{C}$ cannot yet be made: even with a complete set of surface wind and pressure observations, an understanding of boundary layer processes and airflow over

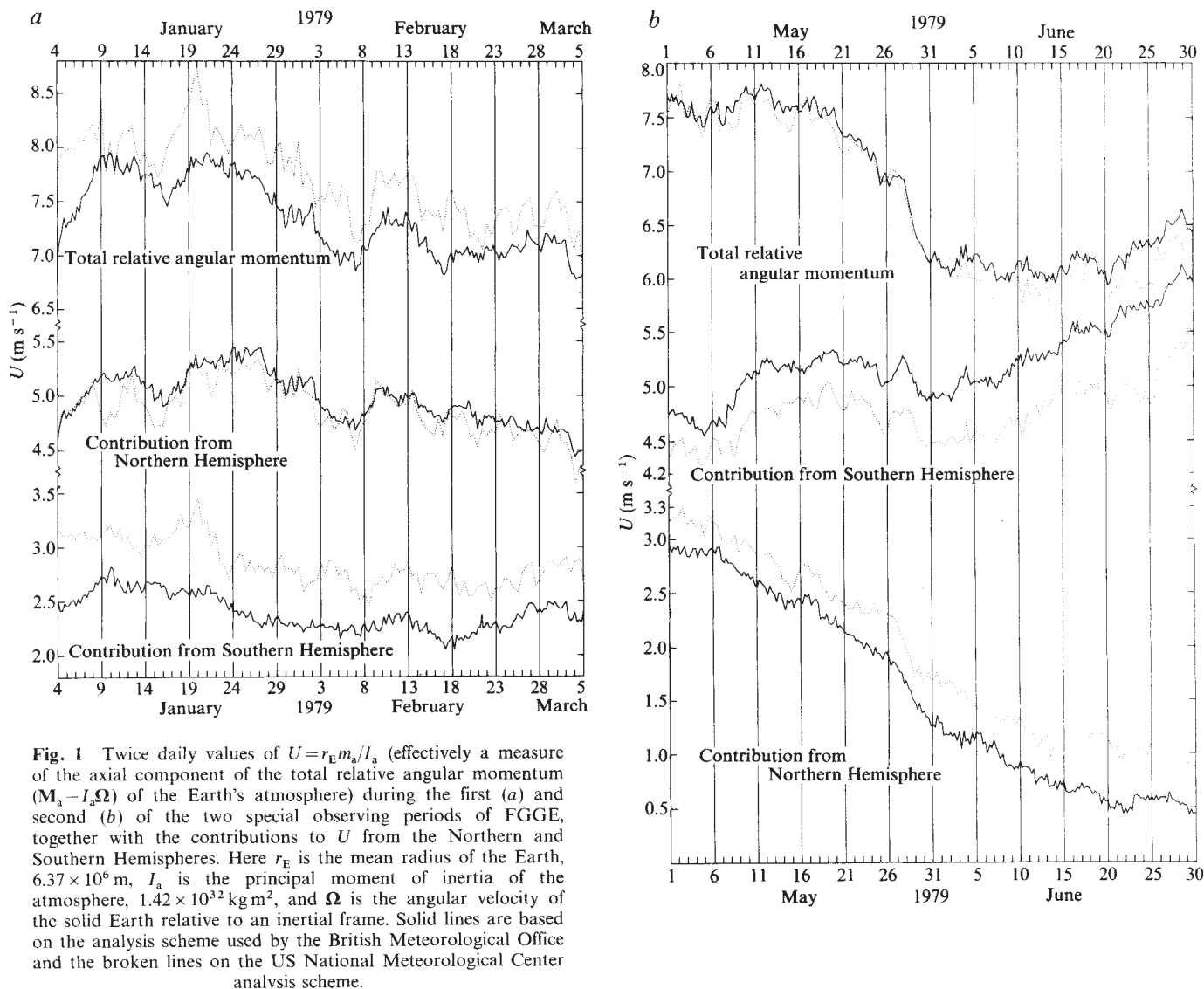


Fig. 1 Twice daily values of $U = r_E m_a / I_a$ (effectively a measure of the axial component of the total relative angular momentum ($\mathbf{M}_a - I_a \boldsymbol{\Omega}$) of the Earth's atmosphere) during the first (a) and second (b) of the two special observing periods of FGGE, together with the contributions to U from the Northern and Southern Hemispheres. Here r_E is the mean radius of the Earth, 6.37×10^6 m, I_a is the principal moment of inertia of the atmosphere, 1.42×10^{32} kg m², and $\boldsymbol{\Omega}$ is the angular velocity of the solid Earth relative to an inertial frame. Solid lines are based on the analysis scheme used by the British Meteorological Office and the broken lines on the US National Meteorological Center analysis scheme.

and around mountain ranges well beyond the present knowledge would be required. But values of C can be inferred indirectly from variations in the distribution and magnitude of zonal winds or from determinations of associated variations in the l.o.d.

Angular momentum budget

In examining the angular momentum budget of the solid and fluid parts of the Earth allowance should be made for the contributions from the oceans and fluid core as well as from the atmosphere. The relative angular momentum associated with ocean currents is very much less than ($\mathbf{M}_a - I_a \boldsymbol{\Omega}$) so that direct angular momentum transfer between the oceans and the solid Earth should produce negligibly small effects. On the other hand the relative angular momentum associated with motion in the liquid metallic outer core might be as much as $10 \times (\mathbf{M}_a - I_a \boldsymbol{\Omega})$ in magnitude, and indeed it is generally considered that angular momentum transfer between the core and solid mantle is responsible for the biggest fluctuations ($\approx 5 \times 10^{-3}$ s) observed in the l.o.d., the so-called 'decade variations'^{1,2,5,6}. It is unlikely (as the present study implies) but not yet certain that the core can produce significant l.o.d. changes on time scales less than a few years; this is a matter for more extensive future investigations of residual effects based on accurate determinations of l.o.d. changes and of the atmospheric contribution⁵. Conversely, the expected relationship between short-period l.o.d. fluctuations and changes in $\mathbf{M}_a$ is likely to involve the moment of inertia I_m

($= 7.1 \times 10^{37}$ kg m²) of the crust and mantle, not the moment of inertia I of the whole Earth, which is $\sim 10^{-10}$ greater than I_m .

The quantity

$$m_a \equiv \int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} \int_0^{2\pi} \int_{r_s}^{\infty} \rho(r, \theta, \lambda, t) u(r, \theta, \lambda, t) r^3 \cos^2 \theta \, dr \, d\lambda \, d\theta \quad (1)$$

is the axial (parallel to $\boldsymbol{\Omega}$) component of the relative angular momentum ($\mathbf{M}_a - I_a \boldsymbol{\Omega}$) of the whole atmosphere. Here ρ and u are the density and zonal (west-east) component of wind velocity at a time t at a point with spherical polar coordinates θ (latitude), λ (longitude) and r (distance from the centre of the Earth), the volume integration being taken over the whole atmosphere, from the surface $r = r_s$ of the Earth upwards. The density and pressure in the atmosphere satisfy the hydrostatic relationship $\partial p / \partial r = -\rho g$ where g is the acceleration due to gravity. Because the effective thickness of the atmosphere is very small compared with the mean radius r_E ($= 6.37 \times 10^6$ m) of the underlying planet, within the atmosphere g does not depart significantly from its mean surface value of 9.81 m s^{-2} and so both r and g can be treated as constants in equation (1). Thus, we have

$$m_a \doteq \frac{r_E^3}{g} \int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} \int_0^{2\pi} \int_0^{p_s} u(p, \theta, \lambda, t) \cos^2 \theta \, dp \, d\lambda \, d\theta \quad (2)$$

where p_s is the surface pressure.

It is convenient to introduce as a measure of m_a a quantity U with dimensions of speed (m s^{-1}):

$$U = r_E m_a / I_a \quad (= 4.48 \times 10^{-26} m_a) \quad (3)$$

U is thus equal to the relative zonal velocity at the equator of a solid body having the same mass distribution and total angular momentum as the atmosphere.

Relative zonal velocity

Determinations of U have been made from data obtained at 12-h intervals during the two periods 4 January (0GMT)–5 March (0GMT) and 1 May (0GMT)–30 June (0GMT) 1979. The procedure adopted is to evaluate U from finite interval approximations to the continuous integral (2) using values of the zonal wind defined at grid points on a global three-dimensional mesh. These grid-point values are obtained from the raw observations (which are, in general, not made at the grid-points) by an elaborate analysis method involving temporal as well as spatial interpolation and weighting based on forecast fields generated by a numerical circulation model⁷. These results are referred to as the Meteorological Office (MO) results. To give a preliminary estimate of the errors involved, the MO results were compared with what we call the NMC results. The latter were obtained by recalculating U from an analysed data set from the US National Meteorological Center: this data set had been produced using the analysis scheme in operation at the NMC⁸. Any discrepancies between the MO results and the NMC results can be mainly attributed to differences in analysis schemes, but also to the fact that the basic data sets were not quite identical. Figure 1a shows the variation of U for the first special observing period as well as the separate contributions from the Northern Hemisphere (NH) and Southern Hemisphere (SH). The difference between the two global variations is largely accounted for by the discrepancy in the SH values, the NH values being in much better agreement. Even in the SH the discrepancy seems to be more systematic than random, so that changes with time are quite well correlated between both

records; however, this is not definite in view of the absence of marked trends in both SH records. The global angular momentum is dominated by the NH contribution. This is expected because of the large pole–Equator temperature gradient in the NH winter, which produces a large vertical variation of zonal winds, and the comparative weakness of mean surface winds. The NH makes a comparatively large contribution to changes in the global angular momentum. This is consistent with the hypothesis that interaction of the energetic winter hemisphere circulation with orography is the main mechanism responsible for fluctuations in U .

Figure 1b shows the variations of U and the contributions from the NH and SH during the second period. Fluctuations in the NH and SH contributions to U in the MO and NMC results are similar, but there are quasi-systematic differences. Thus the NMC results are systematically lower than the MO results for the SH contribution, whereas the reverse is the case for the NH contribution: nevertheless, the two global results are in good agreement. The SH contribution to U dominates during the second period, and the time variation reflects mainly the seasonal decline of the NH contribution. This is associated, of course, with the weakening of the NH zonal circulation as the temperature difference between low and high NH latitudes decreases. Changes on time scales of 1–2 weeks are generally less marked than in the first period, possibly because of the comparative scarcity of land masses and mountain ranges in the SH and the weakness of the NH circulation in summer. However, the most marked change of U found in these results did take place during the second period.

Discussion

A comprehensive error analysis is not yet available; this will form part of future studies. However, the appearance of the same major changes in U in both MO and NMC results presented here suggests that these features are real. A decline in U of $\sim 1 \text{ m s}^{-1}$ between 21 January and 7 February is conspicuous in both MO and NMC results (Fig. 1a) and an even more spectacular decline, $\sim 1.5 \text{ m s}^{-1}$, appears between 18 May and 2 June in both records also (Fig. 1b).

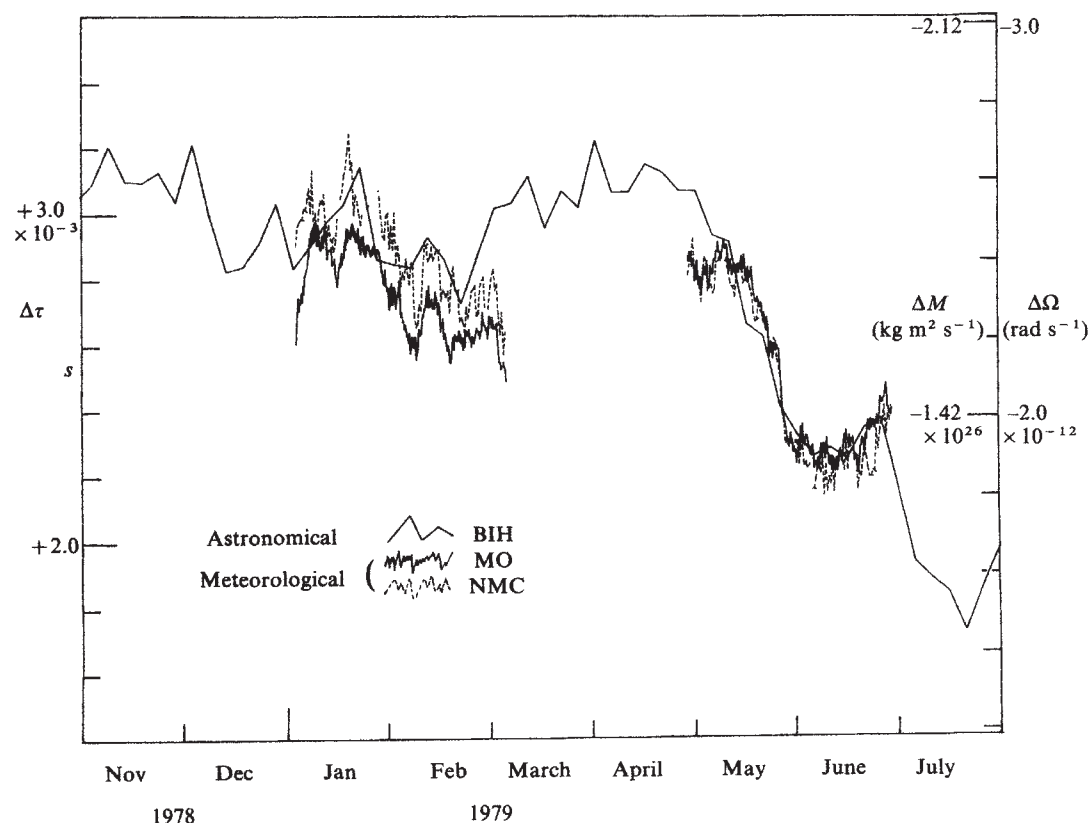


Fig. 2 Comparison of the expected changes in the length of the day ($\Delta\tau$) derived from the meteorological results displayed in Fig. 1 alongside observed changes from astronomical measurements (see text).

We now compare the meteorological results with estimates of l.o.d. variations, as revealed by astronomical observations. These estimates can be made by comparing universal time (UT), defined by the Earth's rotation, with atomic time (TA). UT1 is based on the rate of rotation of the Earth as determined astronomically from transits of stars and Doppler tracking of artificial satellites; TAI is the international atomic time scale based on a certain resonance of the caesium atom and can be regarded as perfectly uniform for the present discussion. It is necessary to allow for all significant tidal effects^{1,2,10}. This was achieved by taking values of the difference between TAI and UT1 at intervals of 5 days as published by the Bureau International de l'Heure (BIH) in Paris, which have already been corrected for annual and semi-annual tides and diurnal nutation, and removing effects due to fortnightly and monthly lunar tides (M_f and M_m (ref. 1)), thus obtaining a quantity $f(n)$, where n is an integer specifying the pentad to which f refers. (The amplitudes of these tides were taken from Table 14.3 of ref. 10.) The values of $f(n)$ were then smoothed to eliminate high-frequency scatter and the first derivative $\Delta\tau(n)$ at each pentad was calculated. This was done using the following 11-point convolute (which exploits the fact that the data were equally spaced in time):

$$\begin{aligned}\Delta\tau(n) \equiv (429 \times 5)^{-1} \{ & 89f(n) + 84(f(n-1) + f(n+1)) \\ & + 69(f(n-2) + f(n+2)) \\ & + 44(f(n-3) + f(n+3)) \\ & + 9(f(n-4) + f(n+4)) \\ & - 36(f(n-5) + f(n+5)) \} \quad (4)\end{aligned}$$

If TAI-UT1 is in seconds, $\Delta\tau(n)$ measures the change in the length of the day relative to the reference day of 86400 SI seconds, which by convention is the average length of the mean solar day during the nineteenth century and corresponds to an angular speed $\Omega_0 = 7.29211515 \times 10^{-5} \text{ rad s}^{-1}$ (see ref. 6). The results are joined by a light continuous line in Fig. 2. The right ordinate gives the corresponding values of $\Delta\Omega(n)$ and $\Delta M(n)$ where

$$\Delta\Omega = (\Omega - \Omega_0) \equiv -\Omega_0^2 \Delta\tau / 2\pi \quad (5)$$

and

$$\Delta M \equiv -\Omega_0^2 I_m \Delta\tau / 2\pi \quad (6)$$

which is the concomitant change $I_m \Delta\Omega$ in the angular momentum of the Earth's mantle.

If the fluctuations δm_a (as measured by δU , see equation (3) and Fig. 1) in the axial component of relative angular momentum ($\mathbf{M}_a - I_a \Omega$) of the atmosphere manifest themselves largely as equal and opposite variations in the axial component of the angular momentum of the mantle of the Earth $I_m \Omega = I_m (\Omega_0 + \Delta\Omega)$ then the corresponding fluctuations $\delta\Omega$ in Ω (and in $\Delta\Omega$ since Ω_0 is constant by definition, see equation (5)) are such that $\delta\Omega$ satisfies

$$\delta\Omega \doteq -\delta m_a / I_m \quad (7)$$

The corresponding fluctuations in the length of the day are given by

$$\delta(2\pi/\Omega) \doteq \delta m_a (2\pi/\Omega_0^2 I_m) \doteq \delta U (2\pi I_a / \Omega_0^2 r_E I_m) \quad (8)$$

Values of the quantity

$$U (2\pi I_a / \Omega_0^2 r_E I_m) \quad (9)$$

are plotted in Fig. 2, based on the MO and NMC results presented in Fig. 1. If the hypothesis expressed by equation (8) is correct and the values of $\Delta\tau$ evaluated by the 11-point

smoothing procedure (see equation (4)) are dependable, then the curves representing the meteorological data in Fig. 2 should resemble the shape of the curve representing the astronomical data but be separated by a constant vertical distance $\Delta\tau_*$, to be determined from the data. Thus

$$\Delta\tau \doteq \Delta\tau_* + U (2\pi I_a / \Omega_0^2 r_E I_m) \quad (10)$$

Comparison of these results shows that this is so, especially during the second special observing period. Indeed the curves are nearly coincident; that is to say $\Delta\tau_* \ll \Delta\tau$. This unexpected result implies that the net reduction of the angular momentum of the mantle due to the combined effects of tidal friction and other agencies (including any couples acting on the base of the mantle as a result of motions the liquid core of the Earth) since the nineteenth century is close in magnitude to the mean relative angular momentum of the atmosphere in 1979.

The likely connection between fluctuations in the zonal winds and in the l.o.d. has long been appreciated¹⁻³ but the earliest studies were handicapped by the incomplete wind data. Now, as a result of FGGE, it has become possible to make a close comparison between the changes in the angular momentum of the atmosphere and the mantle. The agreement overall shown in Fig. 2 between the expected changes in the l.o.d. according to the meteorological results and the changes observed astronomically is good, particularly during the second special observing period. The 11-point smoothing was chosen because it reduced the considerable scatter in the 'raw' astronomical results and yet preserved apparently real fluctuations greater than about 10^{-4} s. The agreement shown in Fig. 2 indicates that the meteorological results provide some control on the degree of smoothing that might safely be applied to the astronomical data. Previously this has only been possible from a consideration of the internal errors, which is an unreliable procedure.

Conclusions

By continuing the surveillance of the atmosphere it should be possible to subtract the changes in the angular momentum of the atmosphere from the astronomically determined changes in the angular momentum of the mantle and thus find the residual changes due to interaction between the mantle and the core. The magnitude and temporal behaviour of this interaction will be of particular interest. When the new methods for monitoring the Earth's rotation based on very long base-line interferometry and laser ranging to artificial satellites and the Moon are fully operational, it should also prove possible to carry out a more detailed comparison with the meteorological data. The results of such an investigation will be reported elsewhere.

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Evidence for live ^{247}Cm in the early Solar System

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Variations of the $^{238}\text{U}/^{235}\text{U}$ ratio in the Allende meteorite, ranging from -35% to $+19\%$, are interpreted as evidence of live ^{247}Cm in the early Solar System. The amounts of these and other r -products in the Solar System indicate values of $(9,000 \pm 3,000)$ Myr for the age of the Galaxy and ~ 8 Myr for the time between the end of nucleosynthesis and the formation of meteoritic grains. Three possible explanations are presented for the different values of the latter time period which are indicated by the decay products of ^{247}Cm , ^{26}Al , ^{244}Pu and ^{129}I .

THE nuclide ^{247}Cm decays to ^{235}U with a half life ($t_{1/2}$) of 15.6 Myr. As both parent and daughter are actinides, the existence of live ^{247}Cm at the time when solids formed in the primitive nebula would only cause variations in the present $^{238}\text{U}/^{235}\text{U}$ ratio if chemical fractionation separated Cm from U. Thus, uranium isotopes in primitive solids may provide important information on the time scale (T) for galactic nucleosynthesis and the time interval (Δ) between the termination of nucleosynthesis and the formation of solids in the early Solar System¹.

Earlier age estimates, based on the normal $^{238}\text{U}/^{235}\text{U}$ ratio, 137.88 (ref. 2), the $^{238}\text{U}/^{232}\text{Th}$ ratio, and/or these ratios in combination with the $^{187}\text{Re}/^{187}\text{Os}$, $^{244}\text{Pu}/^{238}\text{U}$ and $^{129}\text{I}/^{127}\text{I}$ ratios³⁻¹¹, indicate that galactic nucleosynthesis occurred on a time scale T of $\sim 10^4$ Myr and that solids formed after an interval Δ of $\sim 10^2$ Myr. However, recent discoveries of the decay product of ^{26}Al ($t_{1/2} = 0.73$ Myr) in Allende^{12,13} and the decay product of ^{107}Pd ($t_{1/2} = 6.5$ Myr) in the Santa Clara iron meteorite¹⁴ imply that the interval Δ may instead be ~ 1 Myr.

In addition to isotopic anomalies from *in situ* decay of short-lived radionuclides which existed in the early Solar System, the discovery of isotopically anomalous oxygen in Allende was interpreted as evidence that meteorites trapped ^{16}O -enriched interstellar dust grains¹⁵. It has also been suggested that the oxygen anomaly indicates incomplete mixing of material injected from a nearby supernova (SN) which triggered the collapse of the presolar nebula¹⁶, or isotopic differences between the various chemical components of the interstellar medium which were taken up differentially as solids formed¹⁷. The discovery of nucleogenetic anomalies, in addition to radiogenic ones, has overthrown the dogma of isotopic homogeneity in the pre-solar nebula, and raised doubts about the validity of classical nucleocosmochronology.

Recently, Arden¹⁸ reported that the $^{238}\text{U}/^{235}\text{U}$ ratio is as low as 46 in an acid-etched residue of Allende, and additional analyses seemed appropriate to verify these findings. We report here the results of precise measurements of uranium isotopes in various phases of the Allende, C3V carbonaceous chondrite. Variations in the isotopic composition of uranium are attributed to the existence of ^{247}Cm and to chemical fractionation of $^{247}\text{Cm}/\text{U}$ in the early Solar System. The levels of ^{247}Cm indicated by our results are then used in the Cm/U-Th cosmochronometer to consider the SN trigger¹⁶ and the presolar grain^{15,17} hypotheses.

Experimental techniques and standards

The isotopic composition of uranium and concentrations of U and Th in inclusions and acid resistant residues of Allende were precisely determined by using a spike enriched in ^{233}U , ^{236}U and ^{230}Th and by pulse-counting on a two-stage, NBS-type mass spectrometer¹⁹. Details of the present study will be published elsewhere²⁰. The ^{233}U - ^{236}U double-spike was used to monitor mass fractionation of uranium isotopes.

By this technique, the $^{238}\text{U}/^{235}\text{U}$ ratio can be reproducibly determined in 1 ng of standard uranium, NBS-U-500, to within $\pm 0.01\%$ of the certified value. However, in 0.1–1.0 ng of a standard of natural uranium, NBS-950a, this ratio can be determined only to within $\pm 0.5\%$ (2σ). The reproducibility of $^{238}\text{U}/^{235}\text{U}$ ratio measurements on a standard solution of NBS-950a and on terrestrial basalts is shown in Fig. 1. The accuracy of the ratio measurement on small amounts of uranium depends solely on the count rate of ^{235}U , and 0.1 ng of natural uranium always provided a signal of more than 100 counts per second (c.p.s.). When the count rate of ^{235}U is less than 50 c.p.s., the apparent value of the $^{238}\text{U}/^{235}\text{U}$ ratio tends to become smaller because of background contribution to the ^{235}U signal.

Uranium anomalies in chondrules and inclusions

Inclusions of Allende were separated from a specimen coated with fusion crust using stainless steel tools under a laminar flow bench in an ultra-clean laboratory. Inclusions supplied from the National Museum of Natural History and several inclusions left from the previous U/Pb chronology study²¹ were also analysed. Some of the inclusions were analysed by electron microprobe for mineralogy. The isotopic compositions which we measured for U in these inclusions are shown in Fig. 2.

Our results demonstrate that: (1) inclusions of Allende indeed display variations in the $^{238}\text{U}/^{235}\text{U}$ ratio, ranging from -4 to $+3.5\%$ of the terrestrial value, 137.88, whereas Arden¹⁸ reports normal to lower values in all samples except one; (2) spinel-bearing pink aggregates show definite negative isotopic anomalies in the $^{238}\text{U}/^{235}\text{U}$ ratio ranging from -4 to -0.5% , but all white to grey aggregates show positive anomalies ranging from $+0.5$ to $+3.5\%$; and (3) the Mg-rich chondrules show no definite anomaly pattern, with variations of $+3$ to -3% in the $^{238}\text{U}/^{235}\text{U}$ ratio, whereas this ratio ranges from normal to $+2\%$ in the Ca-Al-rich chondrules.

Such large variations in the $^{238}\text{U}/^{235}\text{U}$ ratio (up to 7%) cannot be the result of isotopic fractionation alone and will

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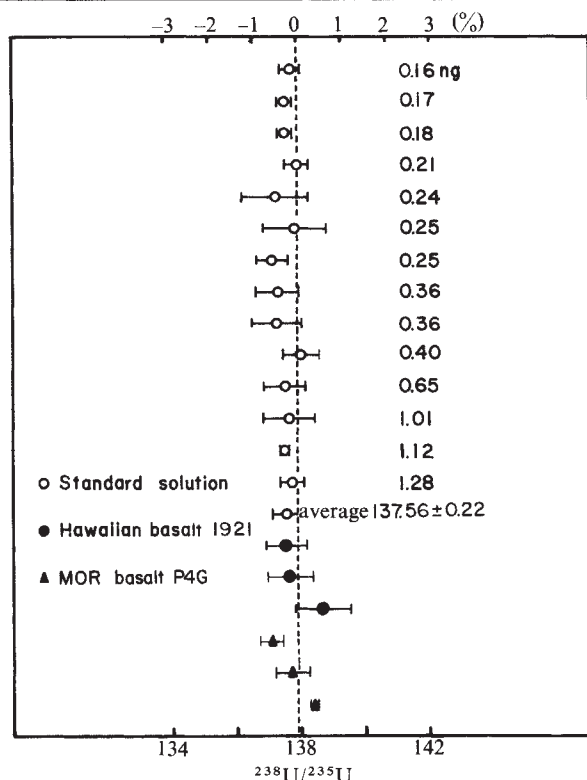


Fig. 1 Reproducibility of $^{238}\text{U}/^{235}\text{U}$ ratio measurements on the NBS-950a standard and on terrestrial basalts.

therefore be taken to indicate Cm/U fractionation. In view of the fact that ^{244}Pu ($t_{1/2}=81$ Myr) and ^{129}I ($t_{1/2}=16$ Myr) survived the interval Δ , it would be reasonable to assume that ^{247}Cm ($t_{1/2}=16$ Myr) was also present in early Solar System condensates. If chemical fractionation of Cm/U occurred, then the grey to white aggregates trapped actinides with a lower Cm/U ratio than did the pink aggregates. Although the mineral components responsible for these fractionations are not known to us, it seems plausible that the orange-red V-rich spinel or Ti-bearing fassaite²² may have lower $^{238}\text{U}/^{235}\text{U}$ ratios because $^{247}\text{Cm}^{3+}$ may have entered V^{3+} or Ti^{3+} crystal sites in preference to UO_2^{2+} .

Alternative explanations for the variations observed in the $^{238}\text{U}/^{235}\text{U}$ ratio include: (1) differences in recoil effects when the U isotopes were produced by α -decay; (2) contributions to the presolar nebula of transbismuth elements from two or more r-processes of nucleosynthesis with different $^{238}\text{U}/^{235}\text{U}$ production ratios; (3) contributions from two or more nucleosynthesis events which occurred at different times; (4) an 'early' cosmic-ray irradiation which preferentially destroyed ^{235}U (ref. 23); or (5) the presence of live ^{242}Pu ($t_{1/2}=0.38$ Myr), the precursor of ^{238}U , in the early Solar System. The latter possibility is indicated if Lee *et al.*²⁴ are correct in concluding that ^{26}Al levels in Ca-Al-rich inclusions of Allende require a very short formation interval after nucleosynthesis, $\Delta < 3$ Myr. In any case, the early Solar System contained other r-products, ^{244}Pu and ^{129}I , with half lives comparable with that of ^{247}Cm . Thus, Cm/U fractionation is the most probable cause for the ^{235}U excess.

Uranium anomalies in acid etched residues

Because the mineral phases responsible for the negative and positive anomalies were not clearly identified in this early phase of our study, we prepared HCl-HF insoluble residues of Allende matrix to see if the anomalous uranium might reside in the minerals (carbon, chromite and spinel) which contain isotopically anomalous noble gases and tellurium²⁵⁻²⁹. The

presence of anomalous noble gases and tellurium in such residues implies a signature of presolar grains which might be the source of isotopically anomalous uranium.

Our procedure for preparing these residues is that of Anders' group²⁵⁻²⁷. After removal of most visible inclusions, an insoluble residue of 154 mg was prepared from 31 g of the Allende matrix by treatment with HCl-HF at room temperature. The residue was then etched repeatedly, for periods of 24 h, in 6 M HCl. Etching was conducted in a Teflon container at 150 °C, with the dissolution of chromite, and probably spinel. After five HCl etchings, the solution was essentially colourless, indicating that the chromite (FeCr_2O_4), and probably the spinel (MgAl_2O_4), were dissolved. We denote this fraction as 'spinel'. The black residue which remained was then etched for 24 hours in concentrated HNO_3 (16 M) at 200 °C. In these conditions, the 'carbon' was dissolved leaving a yellowish-white residue. This residue was further treated with concentrated HClO_4 for one day at 150 °C.

The dissolution of 'spinel' in HCl at elevated temperatures was verified by a mock experiment on terrestrial chromite and spinel. The HCl dissolution was used instead of the H_3PO_4 - H_2SO_4 mixture^{27,29} to minimize analytical blanks. The dissolution of 'carbon' may be accompanied by the dissolution of an ill-defined mineral 'Q' (refs 25-27). We denote this fraction as 'carbon' because it was obviously the dominant phase which dissolved when the residue was treated with concentrated HNO_3 at 200 °C.

Measured concentrations of U and Th in each fraction and the isotopic compositions of U are presented in Table 1. Due to the low ^{235}U count-rate in fraction 2 the data for 'spinel' have large experimental uncertainties. In spite of this, the high $^{238}\text{U}/^{235}\text{U}$ ratio in 'spinel' is quite significant because, as noted above, measured $^{238}\text{U}/^{235}\text{U}$ ratios tend to become small when the ^{235}U signal is less than 50 c.p.s. The amounts of U in fractions 3, 4, 5 and 7 were too low for reliable isotopic analysis.

The $^{238}\text{U}/^{235}\text{U}$ ratio in carbon is low, and the intermediate value of $^{238}\text{U}/^{235}\text{U}$ in fraction 1 is probably due to physical flotation of some extremely fine, black particles which later dissolved when the decantate was converted in an HNO_3 medium for purification of U by anion exchange.

We estimate the $^{238}\text{U}/^{235}\text{U}$ ratio in the total acid residue to be ~ 116 from the values observed in fractions 1, 2 and 6. However, as our residue was prepared from matrix-rich portions, the results of this estimate do not necessarily provide support for the low values reported by Arden¹⁸.

The extremely high value of the $^{238}\text{U}/^{235}\text{U}$ in fraction 2, when 'spinel' dissolved, seems to be inconsistent with Arden's report¹⁸ of extremely low values of this ratio in residues which had been previously exposed to various oxidizing conditions. Regardless of whether HCl+HF or HNO_3 +HF were used in the first etching stage, Arden observed low values of the $^{238}\text{U}/^{235}\text{U}$ ratio, ~ 50 , in the final stage of etching when the residues contained extremely small amounts of uranium, 10^{-11} g.

The most significant results of this etching experiment are: the high value of the $^{238}\text{U}/^{235}\text{U}$ ratio in fraction 2 when 'spinel' dissolved; and the extremely low value of this ratio in fraction 6 when 'carbon' dissolved. Enriched ^{235}U in 'carbon' and its depletion in 'spinel' parallel abundances of the heavy and light isotopes of Xe and Te in these^{25-27,29}. If heavy isotopes of Xe and Te in carbon are from actinide fission, it implies that excess ^{235}U there may be due to an initial enrichment of live ^{247}Cm . These large anomalies are unlikely to be artefacts of the acid treatment, such as the selective extraction of radiogenic ^{235}U from 'spinel' because of α -recoil effects, and its adsorption on 'carbon', for the following reasons: (1) active sites for adsorption of uranium onto carbon would be swamped with uranium of normal composition which was released from the silicates during the earlier HCl-HF treatments; (2) the high $^{238}\text{U}/^{235}\text{U}$ ratio in the spinel-rich

Table 1 Data of Allende HCl-HF residue (154 mg)

Fractions	Amount (cm ³)	U* (p.p.b.)	Th* (p.p.b.)	% Blank U Th	Th U	$\frac{238}{235} \pm 2\sigma_m$	^{235}U (c.p.s.)
1 6 M HCl	20	1.79	17.89	40 32	9.99	123.16 ± 0.66	242-33
2 6 M HCl	20	0.58	3.11	42 45	7.2	163.50 ± 1.26	67-16
3 6 M HCl	20	0.19	2.18	200 76	11.5	—	—
4 and 5 as above	20	—	—	—	—	—	—
6 16 M HNO ₃	10	0.99	1.12	28 200	1.1	89.88 ± 2.79	244-88
7 11 M HClO ₄	2	0.11	0.83	110 135	7.6	—	—

*Concentrations are for total residue; p.p.b., parts per 10⁹. †Blank corrected ratios, errors are 2 × mean standard deviation.

fraction 2 is in accordance with the high values of this ratio in the spinel-rich, white inclusions which were mechanically separated from Allende; and (3) we are unaware of any chemical treatments which can separate the isotopes of such dissimilar elements as uranium, xenon and tellurium to produce the unusual isotopic compositions reported in acid-etched residues of Allende. We will, therefore, assume that variations in the $^{238}\text{U}/^{235}\text{U}$ ratio were produced by the decay of ^{247}Cm in the early Solar System.

The curium-uranium-thorium cosmochronometer

In the following calculations of the duration of galactic nucleosynthesis (T) and the interval Δ , we follow Fowler's³⁰ model of an exponentially decreasing rate of galactic nucleosynthesis with a last minute spike event. Local variations from a smooth exponential decline in the nucleosynthesis rate do not seriously detract from the general versatility of this model³¹. In fact, the last minute spike^{7,8,30} is remarkably similar to recent models of a SN trigger which injected freshly synthesized material into a presolar nebula^{16,32} of elements from earlier, collective nucleosynthesis processes.

If the transbismuth nuclides were produced by an r-process at a different site (core³³?) from the explosive carbon-burning or hydrogen-helium-burning shells where ^{16}O and ^{26}Al were produced³⁴⁻³⁶, then the high $^{238}\text{U}/^{235}\text{U}$ ratio and low concentration of ^{244}Pu in spinel might be explained by early condensation of spinels before mixing with the heavy r-process

nuclides³⁷. In this regard, note that the uranium which we extracted from 'spinel' represents a mixture of uranium from spinel, chromite and other spinels. Thus, the weakness of our experiment is its failure to set a firm upper limit on the value of the $^{238}\text{U}/^{235}\text{U}$ ratio which might be found by selective dissolution of the mineral components in our 'spinel'.

Alternatively, if excess ^{16}O and ^{26}Mg are the result of material injected from a nearby SN^{16,32}, then classical nuclear cosmochemistry may be affected^{33,38,39}. However, the ^{16}O and ^{26}Al may alternatively have been produced locally, in the early Solar System^{40,41}. Moreover, there have been many studies which suggest that isotopic homogeneity, rather than isotopic heterogeneity, was the dominant trait of the presolar nebula, for example, the negative results in attempts to find isotopically anomalous Ca, Sr, Ba and Sm in all Allende inclusions^{24,42} analysed, except two Ca-Al-rich ones, and the finding of isotopically normal Sr and Nd in the HCl-HF etched residues of Allende⁴³. These findings suggest that it would be premature to dismiss classical cosmochemistry.

We will, therefore, use our results to compute values of T and Δ from the Cm-U-Th chronometer and compare these with values of T and Δ suggested by other radionuclides, such as, ^{244}Pu and ^{26}Al . For this calculation, we assume that the value of $^{238}\text{U}/^{235}\text{U}$ of 137.88 in bulk meteorites and terrestrial rocks is characteristic of solar uranium, although microscopic mineral constituents and inclusions of meteorites contain anomalous uranium.

According to Fowler's³⁰ model of exponentially declining nucleosynthesis, the production rate, P_A , of isotope A at time t is $P_A(t) = P_A(t=0) \exp(-\Lambda t)$. Thus, the primordial abundance ratio of isotopes A and B at the time of formation of planetary solids can be expressed as:

$$\frac{N_A(T+\Delta)}{N_B(T+\Delta)} = \frac{P_A/P_B}{\exp\{(\lambda_B - \lambda_A)\Delta\}} \times \frac{S\{1 - \exp(\Lambda T)\} + (1-S)(\Lambda/\Omega_A)\{1 - \exp(\Omega_A T)\}}{S\{1 - \exp(\Lambda T)\} + (1-S)(\Lambda/\Omega_B)\{1 - \exp(\Omega_B T)\}} \quad (1)$$

In these equations, Λ is the decay constant of the assumed exponential form for nucleosynthesis, $\Omega_A = \Lambda - \lambda_A$ and $\Omega_B = \Lambda - \lambda_B$ where λ_A and λ_B are the decay constants of A and B, and S is the fraction of elements in the Solar System that was produced by a spike nucleosynthesis event which occurred at time T , at the termination of galactic nucleosynthesis of Solar System material.

To obtain the chronology of r-process nucleosynthesis from ^{247}Cm -U, the degree of fractionation of Cm/U in Solar System material must be known. However, the fractionation factor for ^{247}Cm at $T+\Delta$ is unknown. For simplicity, we therefore assume, in the first case, that the addition of freshly synthesized ^{247}Cm and U in the spike resulted in the present value of N_{235}/N_{238} is 1/137.88 in the Earth and in many bulk meteorites for unfractionated Cm/U abundances, and that the present value of N_{235}/N_{238} of 1/163 in 'spinel' represents the base value for the most extreme Cm/U fractionation, that is, this represents the value when there has been no ^{247}Cm contribution to 'spinel' uranium. Accordingly, the amounts of

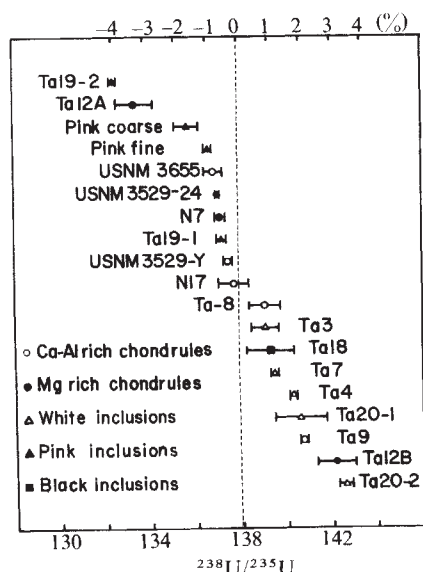


Fig. 2 Uranium isotopic compositions in the Allende inclusions. Pink aggregates, N7 and N17, were described earlier²¹. USNM samples are from the National Museum of Natural History.

^{247}Cm , ^{235}U and ^{238}U in the early Solar System were, assuming the meteorite age to be 4.55×10^3 Myr:

$$\frac{N_{235}^G(T+\Delta) + N_{235}^S(T+\Delta) + N_{247}^G(T+\Delta) + N_{247}^S(T+\Delta)}{N_{238}^G(T+\Delta) + N_{238}^S(T+\Delta)} = (1/137.88) \exp \{(\lambda_{235} - \lambda_{238})(4.55)\} = 0.3163 \quad (2)$$

and the ^{247}Cm -free ratio of $^{235}\text{U}/^{238}\text{U}$ was

$$\frac{N_{235}^G(T+\Delta) + N_{235}^S(T+\Delta)}{N_{238}^G(T+\Delta) + N_{238}^S(T+\Delta)} = \{1/163\} \exp \{(\lambda_{235} - \lambda_{238})(4.55)\} = 0.2675. \quad (3)$$

In these equations, the superscripts G and S designate contributions from galactic and spike nucleosynthesis, respectively. Then for this case, the ratio of ^{247}Cm to ^{238}U at time $T+\Delta$ is

$$\frac{N_{247}^{G+S}(T+\Delta)}{N_{238}^{G+S}(T+\Delta)} = 0.3163 - 0.2675 = 0.0487. \quad (4)$$

For the above model we have three input data which are associated with the r-process, $^{247}\text{Cm}/^{238}\text{U}$, $^{235}\text{U}/^{238}\text{U}$ and $^{232}\text{Th}/^{238}\text{U}$. These were used to determine the values of T , Δ , Λ and S , using the values of constants listed in Table 2. For values of $\Lambda = (0.1 \text{ to } 1.0) \times 10^{-9} \text{ yr}^{-1}$ and $S = 0.01 \text{ to } 0.1$, we found that the most internally consistent solution is obtained for $\Lambda = 0.6 \times 10^{-9} \text{ yr}^{-1}$ and $S = 0.05$. Curves of Δ versus T for $\Lambda = 0.6 \times 10^{-9} \text{ yr}^{-1}$ are shown in Fig. 3. This solution yields a galactic age, $T_G = (T + \Delta + 4.550 \text{ Myr}) = 9,100 \pm 2,800 \text{ Myr}$, (assuming $\pm 10\%$ uncertainty in production rates and $\pm 5\%$ uncertainty on the Th/U ratio), and a decay interval $\Delta \sim 8 \text{ Myr}$, with limits of 0–90 Myr for the above uncertainties.

Detailed calculations including the constraints imposed by the abundances of ^{244}Pu , ^{129}I and ^{26}Al will be presented elsewhere²⁰. However, note that the $^{244}\text{Pu}/^{238}\text{U}$ curve intersects the $^{235}\text{U}/^{238}\text{U}$ (1/163) and $^{232}\text{Th}/^{238}\text{U}$ curves at $\Delta \simeq 100 \text{ Myr}$, if the $^{244}\text{Pu}/^{238}\text{U}$ ratio at $t = T + \Delta$ is 0.016 (ref. 44) or 0.035 (ref. 45). However, the curve for $^{244}\text{Pu}/^{238}\text{U} = 0.087$, which Podosek and Lewis⁴⁶ observed in a Ca–Al-rich Allende inclusion crosses the curves of $^{235}\text{U}/^{238}\text{U}$ and $^{232}\text{Th}/^{238}\text{U}$ at $\Delta = 8 \text{ Myr}$. These calculations are based on values of $\Lambda = 0.6 \times 10^{-9} \text{ yr}^{-1}$ (see Fig. 3).

The assumption that the $^{238}\text{U}/^{235}\text{U}$ ratio in 'spinel' contains a negligible contribution from spike-produced ^{247}Cm but that 5% of other r-products in spinels came from this last nucleosynthesis spike, may not be correct if spinels formed before contamination with the spike r-products. However, note that the value which we calculated for the initial $^{247}\text{Cm}/^{238}\text{U}$ ratio, 0.049, is the same order of magnitude as values reported for the initial $^{244}\text{Pu}/^{238}\text{U}$ ratio, 0.016–0.087 (refs 44–46).

The value calculated above for the decay interval, $\Delta \sim 10 \text{ Myr}$, is somewhat higher than the value of $\Delta < 3 \text{ Myr}$ which Lee *et al.*²⁴ obtained from data indicating that $^{26}\text{Al}/^{27}\text{Al} \simeq 5 \times 10^{-5}$ when Ca–Al-rich inclusions of Allende formed. Note, however, that Lorin and Christophe Michel-Lévy⁴⁷ found evidence of much higher initial $^{26}\text{Al}/^{27}\text{Al}$ ratios, up to 1×10^{-3} , in hibonites in inclusions of Leoville and Allende. Recently Lee *et al.*⁴⁸ reported a very low initial $^{26}\text{Al}/^{27}\text{Al}$ value of 2×10^{-7} in HAL inclusion of Allende. The difference between initial values of $^{26}\text{Al}/^{27}\text{Al}$ in hibonites of Leoville and in HAL inclusion of Allende corresponds to an interval of 9 Myr between the formation times of these objects⁴¹, if the source of Al in these objects originally had the same $^{26}\text{Al}/^{27}\text{Al}$ ratio. This suggests that hibonite grains formed directly from freshly synthesized material, or very soon after this material was added to the presolar nebula, and that solidification of 1–10-mm size objects occurred $\sim 10 \text{ Myr}$ later.

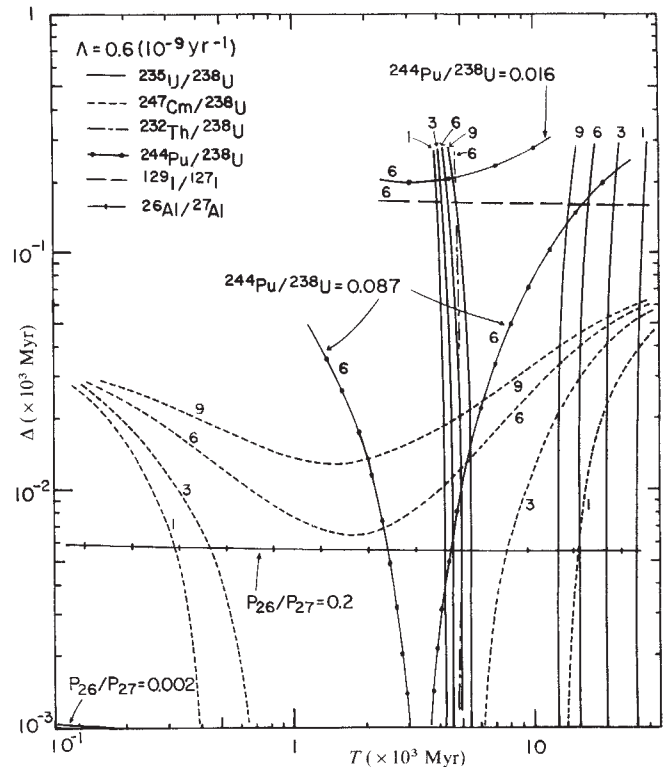


Fig. 3 Trajectories of Δ and T values of the $^{235}\text{U}/^{238}\text{U}$, $^{232}\text{Th}/^{238}\text{U}$, $^{247}\text{Cm}/^{238}\text{U}$, $^{244}\text{Pu}/^{238}\text{U}$ and $^{26}\text{Al}/^{27}\text{Al}$ ratios for $\Lambda = 0.6 \times 10^{-9} \text{ yr}^{-1}$ of case 1. They are calculated following Fowler's model³⁰ of exponential galactic nucleosynthesis [$\exp(-\Lambda T)$] and a last minute spike (S). Numbers on the trajectories are S (%). For the trajectories of $^{232}\text{Th}/^{238}\text{U}$, $^{244}\text{Pu}/^{238}\text{U}$, $^{129}\text{I}/^{127}\text{I}$ and $^{26}\text{Al}/^{27}\text{Al}$, only values corresponding to $S = 6\%$ are shown. Production ratios assumed for P_{26}/P_{27} are 0.002 and 0.2, and values assumed for the $^{244}\text{Pu}/^{238}\text{U}$ ratio at time $T + \Delta$ are 0.087 and 0.016.

Conversely, these results may suggest that the production ratio of $^{26}\text{Al}/^{27}\text{Al}$ from some thermonuclear process in the SN was about two orders of magnitude higher³⁶ than that calculated for carbon burning^{35,49}.

For the second case, we assume that there was no contribution of any transbimuth r-products from the spike to 'spinel'. In this model, we assume that the 'spinel' represent grains which formed in the outer shell of the SN³⁷ and that these were injected into the presolar nebula, where the average mixture of all r-products is represented by the present value of $(^{238}\text{U}/^{235}\text{U}) = 137.88$ when no fractionation of Cm/U occurred.

This can be described by analogous forms of the equations used for case 1,

$$\frac{N_{235}^G(T+\Delta) + N_{247}^G(T+\Delta)}{N_{238}^G(T+\Delta)} = 0.2675. \quad (5)$$

If we assume that $N_{238}^G(T+\Delta) \simeq N_{238}^G(T+\Delta) + N_{238}^S(T+\Delta)$, then

$$\frac{N_{235}^S(T+\Delta) + N_{247}^S(T+\Delta)}{N_{238}^G(T+\Delta)} = 0.0487. \quad (6)$$

Because ^{247}Cm decays to ^{235}U , the numerator of equation (6) does not change during the period of free decay and cannot be used to calculate the value of Δ . However, if there is negligible

Table 2 Values of constants used in computations

Decay constants (10^{-9} yr^{-1})			
λ_{247}	43.3	λ_{244}	8.41
λ_{235}	0.98485	λ_{129}	41.0
λ_{238}	0.155125	λ_{26}	950
λ_{232}	0.04948		
Abundance ratios (at $T + \Delta$)			
$^{247}\text{Cm}/^{238}\text{U}$	0.0487 ± 0.0267	$^{244}\text{Pu}/^{238}\text{U}$	0.087 ± 0.016
$^{235}\text{U}/^{238}\text{U}$	0.2675 ± 0.027	$^{129}\text{I}/^{127}\text{I}$	$(1.07 \pm 0.41) \times 10^{-4}$
$^{232}\text{Th}/^{238}\text{U}$	$2.48 \pm 0.12^*$	$^{26}\text{Al}/^{27}\text{Al}$	5×10^{-5}
Production ratios			
P_{247}/P_{238}	$0.837 \pm 0.126^\dagger$	P_{244}/P_{238}	$0.90 \pm 0.1^{\#}$
P_{235}/P_{238}	$1.64 \pm 0.25^\ddagger$	P_{129}/P_{127}	$1.52 \pm 0.5^{\#}$
P_{232}/P_{238}	$1.80 \pm 0.1^{\#}$	P_{26}/P_{27}	$2 \times 10^{-3}^{**}, 0.2^\dagger$

* $(^{232}\text{Th}/^{238}\text{U})_{\text{now}} = 4.0 \pm 0.2$. † ref. 3; ‡ ref. 46; $^{\#}$ ref. 44; $||$ ref. 55; ¶ ref. 24; $^{\#}$ ref. 56; ** ref. 49; † ref. 36.

decay of ^{235}U , ^{238}U and ^{232}Th over the time period Δ , that is, $\lambda_{235}, \lambda_{238}, \lambda_{232} \ll 1/\Delta$, then $N_{235}^{G+S}(T+\Delta) = N_{235}^{G+S}(T)$, and so on, and if we assume that $N_{232}^{G+S}(T) \approx N_{232}^G(T)$, then equation (1) yields $T_G = (9.4 \pm 2.7) \times 10^9 \text{ yr}$, $\Lambda = 0.45 \times 10^{-9} \text{ yr}^{-1}$ and $S = 0.02$ for case 2 (where $S=0$ for all r-products in 'spinel').

If this model is correct, then the Cm/U ratio in 'carbon' has been increased by a fractionation factor of ≈ 4 . Such a fractionation factor is quite reasonable from thermodynamic considerations⁵⁰. We prefer case 2, where neither Cm nor U from the spike contributed to 'spinel', because case 1 requires a large fractionation of spike-produced Cm/U in 'spinel'. The duration of galactic r-process nucleosynthesis from our calculations, although model dependent, seems to be consistent with the revised age of the Universe which was recently reported from a new calculation of the Hubble constant⁵¹.

For case 2, we assumed that grains of 'spinel' are fresh condensates from the triggering SN event and that this event also contributed transbismuth r-products to the presolar nebula. On the other hand, Lattimer *et al.*³² note that the difference between values of the decay interval indicated by radiogenic Xe isotopes ($\Delta \sim 100 \text{ Myr}$) and Mg isotopes ($\Delta \sim 1 \text{ Myr}$) may imply that the last SN event was a source of ^{26}Al , but not a source of r-products. According to this hypothesis, the presolar nebula consists of interstellar type gas and dust which had been enriched in r-products when the

nebula passed through a galactic spiral arm⁵² $\sim 100 \text{ Myr}$ before this last SN event. If interstellar material had been cycled, by star formation and stellar ejections, with such a frequency³⁴ that the mean residence time of material in the interstellar medium is short, $< 100 \text{ Myr}$, then our calculations are compatible with the proposal of Lattimer *et al.*³². However, our calculations would not be warranted if the residence time of interstellar material is large, $> 1,000 \text{ Myr}$, as estimated by Clayton¹⁷.

Conclusion

The Cm–U–Th chronometer yields values of $T = 9,000 \pm 3,000 \text{ Myr}$ and $\Delta \sim 8 \text{ Myr}$. This value of Δ is larger than that implied from the decay product of ^{26}Al , $\Delta < 3 \text{ Myr}$, but smaller than that suggested by the decay products of ^{244}Pu , $\Delta \sim 100 \text{ Myr}$.

We suggest three possible explanations for the different values of Δ . (1) The short-lived r-products were produced on the last passage of the protosolar cloud through a galactic spiral arm⁵², $\sim 10 \text{ Myr}$ before ^{26}Al was synthesized³² in the explosion of a nearby SN which triggered the collapse of the presolar nebula¹⁶. (2) Both ^{26}Al and the short-lived r-products were produced in the nearby SN but Al condensed from material in outer shells of the SN $\sim 10 \text{ Myr}$ before the condensation of r-products, ^{247}Cm and ^{244}Pu , from the stellar interior³³. Retention of Xe generally started $\sim 100 \text{ Myr}$ later, except in such cases as the Ca–Al-rich inclusion of Allende for which the ^{244}Pu – ^{136}Xe chronometer indicates $\Delta \approx 8 \text{ Myr}$ (ref. 46). (3) The $^{26}\text{Al}/^{27}\text{Al}$ production rates were different in different regions of the nearby SN, being higher by a factor of ~ 100 in H–He layers³⁶ than in that of carbon burning³⁵. In this case, the observation of $^{26}\text{Al}/^{27}\text{Al} = 5 \times 10^{-5}$ would correspond to a value of $\Delta \sim 6 \text{ Myr}$ (see Fig. 3).

Alternatively, the primitive solar nebula may have contained large scale elemental and isotopic heterogeneities from the debris of a single SN which produced the bulk of the elements in the Solar System⁵³. Note also that the $^{238}\text{U}/^{235}\text{U}$ ratio may have been altered by an early cosmic ray irradiation^{23,54}. If so, then the calculations reported here, and previously calculated values of T and Δ , are unwarranted.

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The influence of codon context on genetic code translation

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A class of mutations that increase the efficiency of a suppressor tRNA in translating a particular amber codon has been characterized. The increased efficiency is due to a mutation resulting in a change in the mRNA that affects the nucleotide adjacent to the 3' side of the UAG triplet. Thus the interaction of tRNA with mRNA is influenced by mRNA sequences outside the triplet codon.

THE triplet words of the genetic code are known to be read by transfer RNA molecules which interact with the respective codons on the messenger RNA. The specificity and accuracy of reading are overwhelmingly due to standard base-pairing between three bases on the mRNA (the codon) and three bases in the tRNA (the anticodon). It is generally assumed that no additional nucleotides in the mRNA other than the three residues which make up a codon are involved in the interaction with a single tRNA species. In the past few years, however, several studies have suggested that reading of a given codon may be influenced by mRNA sequences external to this codon; this influence has been described as the effect of the reading context on translation¹⁻⁸. We have closely investigated this aspect of the decoding process and present here direct evidence that mRNA sequence outside of the codon has a role in determining the efficiency of translation.

Mutant tRNAs, acting as informational suppressors, are tRNA species whose activity can be followed individually *in vivo*. In the appropriate genetic system, the measurement of suppression efficiency can be correlated directly with the activity of the suppressor tRNA. It is then possible to analyse various factors that can influence the activity of the tRNA, such as structural alterations of ribosome, tRNA or mRNA. This approach led us to study the effects of a mutation affecting tRNA structure (*hisT*) on the suppression properties of informational suppressors in *Salmonella typhimurium*.

The *hisT* mutation causes the lack of pseudouridine from the anticodon region of several tRNAs⁹⁻¹². In spite of its pleiotropic effects on many tRNA species, the *hisT* mutation does not impair cell viability. The only detectable phenotypic change is the altered regulation (constitutive expression) of the histidine operon, consequent to the lack of two pseudouridine residues from the anticodon region of histidine tRNA⁹. During our study, it became evident that the activity of the glutamine-inserting amber suppressor *supE*¹³ (which normally contains two pseudouridine residues in the anticodon region^{12,14,15}) is reduced considerably by *hisT*¹⁶ (unpublished results). The reduced efficiency of the suppressor offered the basis for the genetic selection of mutations increasing the efficiency of *supE*. Mutants were isolated in which the inhibitory effect of *hisT* on suppression is overcome by an increase in the efficiency of the translation step at the nonsense site. Interestingly, some of these mutations affect the reading context at the nonsense site by changing the mRNA sequence adjacent to the nonsense codon.

Effect of *hisT* on *supE* suppression efficiency

The ability of *supE* to suppress amber mutations in the *hisD* gene of the *his* operon was tested in both *hisT*⁺ and *hisT*⁻ genetic backgrounds. These tests show that at some mutant

sites the *hisT* mutation causes a reduction in *supE* efficiency so severe that the mutants are not phenotypically corrected by *supE* in the presence of a *hisT* mutation. For some other mutations, however, suppression by *supE* still occurs in spite of the presence of a *hisT* mutation. Thus, the effect of *hisT* on *supE* efficiency seems to depend on the particular mutant site being read. A detailed description of these experiments will be published elsewhere.

We considered three possible explanations for the site-specificity of the effects of *hisT*. First, some mutant sites may yield a protein with poor activity when suppressed by *supE*. Such sites may require a fully active *supE* tRNA in order to show phenotypic correction. Second, some mutant sites may show strong polarity effects. These sites might require very efficient suppression in order to overcome the tendency to terminate mRNA production. Third, some amber codons may be poorly read by a suppressor tRNA due to the influence of the reading context. That is, the efficiency of the suppressor tRNA in reading a particular site may depend on the nature of the residues in the immediate neighbourhood of that site. UAG codons located in unfavourable reading contexts would require a strong suppressor to overcome these reading difficulties. Such context effects on suppression efficiency have been reported previously¹⁻⁷.

Each of these explanations is, in principle, consistent with the initial data presented above if the *hisT* mutation impairs the function of *supE* tRNA. However, there are compelling reasons, outlined below, for rejecting the first two hypotheses.

The first explanation seems untenable because some of the amber sites whose suppressibility is impaired by *hisT* affect the N-terminal portion of the *hisD* protein, which is dispensable for catalytic activity^{17,18} (unpublished results of T. Kohno, I. Hoppe and J.R.). It seems unlikely that the nature of the amino acid insertion in this region could seriously affect protein function. This possibility is further contradicted by the behaviour of amber mutation *hisD6404*. As described below, this site has a glutamine residue in the wild-type sequence. The *hisT* mutation impairs the ability of *supE* (a glutamine-inserting suppressor) to suppress *hisD6404*. Thus the *hisT* effect is seen even though the suppressed protein is wild type in structure.

The second hypothesis implies that the *hisT* inhibition of suppression would have greater effects on the correction of strongly polar mutant sites. This seems unlikely in view of the genetic map distribution of sites whose suppression is strongly affected by *hisT*. In general, the polarity effect of nonsense mutations is a function of their position in the gene, with more polar mutations located nearer the promoter-proximal end of the gene¹⁹. The mutations we have studied show no correlation between map position and effect of *hisT* on their suppressibility by *supE* (data not shown).

Our initial observations are consistent with the idea that reading of a particular amber codon is influenced by the sequence of bases surrounding that codon in the mRNA

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(context). The variable effects of *hisT* on suppressibility of different amber mutations might depend on differences in the context of the UAG codons at the various sites. Several possible mechanisms for these effects are discussed later.

If mRNA context influences the readability of a particular codon, then reading efficiency should be altered if the base sequence near that codon is changed. We did this by selecting mutations that improve the suppression efficiency of *supE* for a particular amber site. The dispensable (N-terminal) region of the *hisD* gene offered a convenient system, because the sequence changes in this region that could affect suppression efficiency were not expected to influence the catalytic activity of the suppressed protein.

Isolation of context mutants

In order to select context mutants, a strain was constructed which carries *hisT*, *supE* and a particular amber mutation in *hisD* whose suppression is abolished by *hisT*; such a strain is a histidine auxotroph in spite of the presence of the suppressor. His⁺ revertants were isolated and screened for individuals that still contained all the parental genetic markers, but had acquired an additional mutational change within the *hisD* gene that restored suppressibility to the amber mutation (change in reading context).

The full genotype of the strain used (TT4242) and the results of the selection are shown in Table 1. The starting amber mutation is *hisD6404*, mapping in the operator-

proximal, dispensable region of the *hisD* gene. Mutation *hisD1242* is a regulatory mutation that causes the *his* operon to be expressed constitutively at high levels^{16,20}; the presence of this regulatory mutation eliminates any further effect of *hisT* on operon expression. Mutation *leu-414* is an amber mutation in the *leu* operon whose suppression by *supE* is also abolished by the presence of a *hisT* mutation. As Table 1 shows, the presence of *leu-414* considerably facilitated the search for context mutants in the initial screening. All the His⁺ revertants arising due to other events (reversion of *hisT*, occurrence of other nonsense suppressor mutations, or any mutation relieving the negative effect of *hisT*) became simultaneously His⁺ and Leu⁺. Table 1 shows that this class included the majority of revertants.

The context mutants were sought among the His⁺ Leu⁻ revertants and had to be distinguished only from revertants of the *hisD6404* which had lost the nonsense mutation. This screening could be performed at an early stage of mutant isolation, due to a morphological property of colonies of *Salmonella* strains derepressed for the histidine operon. Such colonies show a wrinkled morphology when grown on 2% glucose, due to interference with cell division caused by high concentration of the *hisH* and *hisF* gene products²¹. When a derepressed operon contains a polar mutation located operator-proximal to *hisH* and *hisF*, the reduced expression of these genes prevents the wrinkled morphology. When this mutation reverts to prototrophy (His⁺), the morphology of the revertant colonies can be either wrinkled (if the reversion event removes the polarity block), or smooth (if the reversion is due to the activity of a suppressor). (Suppression efficiency is usually insufficient to eliminate polarity completely.) In our case, because of the presence of the regulatory mutation *hisD1242*, the true revertants of *hisD6404* appeared as wrinkled colonies on the selection plates and could be distinguished from smooth-colony revertants whose His⁺ character was due to the activity of *supE*. In Table 1 only the smooth His⁺ Leu⁻ revertant colonies are reported, all of which therefore are due to increased suppression and are potential context mutants.

A more direct genetic test was carried out on the putative context mutants to verify the continued presence of the *hisD6404* amber mutation. Phage lysates were prepared on the revertants using the generalized transducing bacteriophage P22. The lysates were used to transduce isogenic recipient strains carrying a *his* operon deletion, with and without suppressor *supE*. Because this deletion in the recipient strains completely removes the *hisD* gene, donors carrying the *hisD* mutation can give His⁺ transductants only with a recipient strain carrying the amber suppressor, *supE*. Results are shown in Table 2. All the six potential context mutants (hereafter designated *hisD6404-C1,2* and so on) give transductants only with the *supE*-carrying recipient. Thus they still contain an amber mutation; DNA sequencing (see later) shows this to be the original *hisD6404* mutation. Furthermore, when the context variants of *hisD6404* were transduced into a recipient also carrying a *hisT* mutation, His⁺ transductants were seen. This shows that the mutation which relieves *hisT* inhibition of suppression, is present on the same transducing fragment as mutation *hisD6404*.

Other genetic crosses were carried out to rescue *hisD6404* from the *hisD6404-C* (context) revertants. These experiments indicated that the mutations improving *supE* suppression map in the *hisD* gene, very close to *hisD6404* (data not shown).

DNA sequence of *hisD6404* and its context variants

The first structural genes of the histidine operon (*hisG* and *hisD*) have been cloned in both orientations on the single-stranded DNA phage M13 by Barnes²². The resulting M13Hol transducing phages make it possible to determine the DNA sequence using the method of Sanger *et al.*^{23,24} (W. M. Barnes, manuscript in preparation). Therefore, to determine

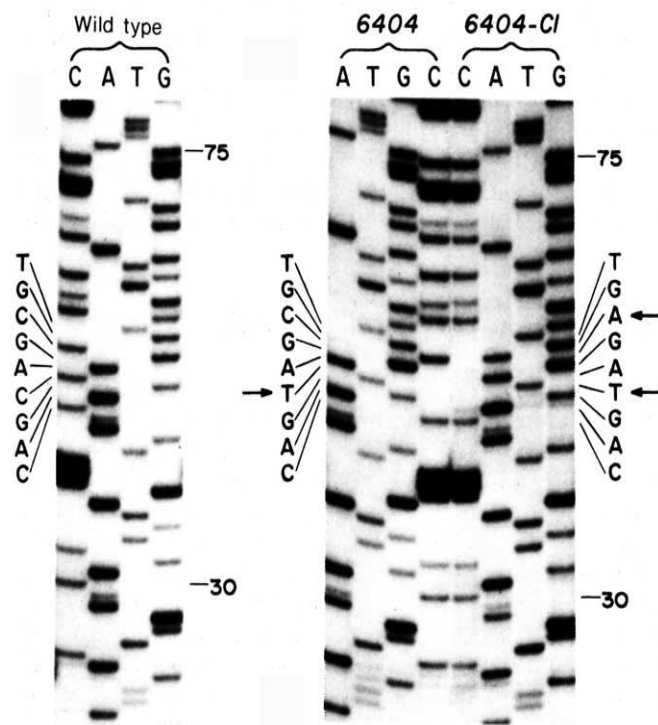


Fig. 1 DNA sequence of *hisD6404* and of its context change. The mutations *hisD6404* and *hisD6404-C1* were placed on M13H0176 (ref. 22) using a genetic procedure (our work in preparation; and unpublished results with H. M. Johnston and S. Lam) which involved the isolation of a Tn5-generated deletion in the *hisG* gene of the transducing phage and subsequent recombination between the phage and a chromosomal *his* region carrying the *hisD* mutations. Single-stranded phage DNA was prepared according to Barnes²². The DNA sequence was determined by the procedure described by Sanger *et al.*²³. Because the orientation of the *his* region present on M13H0176 is opposite to that on the phage genes, the sequencing gels directly give the *hisD* message sequence. The autoradiograms shown from left to right represent the sequences of wild type, *hisD6404* and *hisD6404-C1*. Two arrows indicate the amber and the context mutations, respectively.

Table 1 Isolation of mutants with improved suppressibility of *hisD6404_{am}*

Revertant phenotype	Revertants per 4×10^8 cells
His ⁺ Leu ⁺	105
His ⁺ Leu ⁻	6

The genotype of the parent strain TT4242 is:

*his01242 hisD6404_{am} leu414_{am} supE20 hisT1504 zee-636::Tn5**

Cells of TT4242 were grown to stationary phase, and spread (2×10^8 cells per plate) on minimal medium supplemented with 0.3 mM leucine. Spontaneous His⁺ revertants were isolated and scored for their leucine requirement. Of the His⁺ Leu⁻ class only the smooth colonies were saved (see text). The numbers given represent the combined revertants from two cultures.

**zee-636::Tn5* designates a transposable kanamycin-resistance element (Tn5) co-transducible with the *hisT* locus.

Table 2 Context mutants retain the *hisD6404* mutation

Strain no.	Relevant genotype recipients	His ⁺ transductants with indicated donor strain	
		<i>hisD</i> genotype of donor:	
		6404 (TT4242)	6404-C1 (TT4265)
TT522	<i>hisΔ</i>	—	—
TT3157	<i>hisΔ supE</i>	+	+
TT3138	<i>hisΔ supE hisT</i>	—	+

Transducing phage P22 was grown on strain TT4242 and on its six His⁺ Leu⁻ revertants. The phage lysates were used to transduce the three recipient strains:

TT522 (*his0-F644 zee-1::Tn10**)

TT3137 (*his0-F644 zee-1::Tn10* leu-414_{am} supE20 zee-636::Tn5†*)

TT3138 (*his0-F644 zee-1::Tn10* leu-414_{am} supE20 zee-636::Tn5† hisT1504*)

His⁺ transductants were selected on minimal medium supplemented with 0.3 mM leucine (+ indicates that His⁺ recombinants were observed). All the potential context mutants (isolates 1–6) showed the same behaviour as isolate 6404-C1 (shown above).

**zee-1::Tn10* designates a transposable tetracyclin-resistance element (Tn10) co-transducible with the histidine operon.

†*zee-636::Tn5* designates a transposable kanamycin-resistance element (Tn5) co-transducible with the *hisT* locus.

the DNA sequence of *hisD6404* and of the *hisD6404-C* variants, these mutations were placed by recombination onto M13Hol 76 (one of the M13Hol phages²²). The availability of a detailed restriction map of the *hisG-hisD* region of the *his* operon (Barnes, manuscript in preparation) and of a fine structure genetic map of the *hisD* gene (I. Hoppe and J.R., unpublished results) enabled us to choose a convenient restriction fragment as a primer in the sequencing procedure. Autoradiograms of the sequencing gels for the *hisD* region where the mutations are located are shown in Fig. 1. The inferred mRNA sequences between nucleotides 46 and 54 of the *hisD* gene are shown for wild type, for *hisD6404* and for *hisD6404-C1*, respectively, in Table 3. The result confirms the context hypothesis. The amber mutation *hisD6404* transforms a glutamine codon, CAG, into an amber codon, UAG. The context mutation which permits suppression by *supE* (*hisT*⁻) generates a change in the residue following the UAG codon, a substitution of adenine for cytosine. All six context mutants (which included two independent groups of isolates) were sequenced. All have the same mutational change as *hisD6404-C1* (data not shown). Thus *hisT* inhibition of *supE* activity at this site can be reversed by changing the reading context of the suppressed nonsense codon.

Basis of the context effects

Initially, two general mechanisms were considered to explain the effect of the context mutation on suppression. First, the

hisD6404 UAG codon may be in a context which requires the presence of pseudouridine in *supE* tRNA (*hisT*⁺) for the codon to be read efficiently. This suggests that pseudouridine residues in the anticodon region of *supE* tRNA are only essential for reading UAG in certain contexts. By altering context (as in mutant *hisD6404-C1*) one might generate a site which could be read by *supE* tRNA in the absence of the pseudouridine modification. Second, the *hisD6404* UAG codon may be poorly read even by a normal, fully modified suppressor tRNA (*hisT*⁺). Lack of pseudouridine may impair the ability of *supE* tRNA to read all UAG sites regardless of context effects. This reduction in tRNA efficiency would be critical only at sites that were already poorly read by normally modified suppressor tRNA. The context change might improve readability of the amber codon sufficiently to overcome the *hisT* effect and to provide phenotypic correction.

The two possibilities can be distinguished by quantifying the effect of the context change on the efficiency of *supE* when the suppressor tRNA is fully modified (*hisT*⁺). The first hypothesis predicts that the context mutation would only improve efficiency of a *supE* tRNA lacking pseudouridine in the anticodon region (*hisT*⁻). The second hypothesis predicts that the context change would affect the efficiency of normally modified *supE* tRNA (*hisT*⁺) as well. We have measured the effects of context on *supE* efficiency in both conditions (Table 4). It is clear that codon context strongly affects the activity of *supE* even when the suppressor tRNA is fully modified (*hisT*⁺); the efficiency of *supE* in reading the same UAG codon varies over a 10-fold range depending on the nature of the base adjacent to the 3' side of the codon. This suggests that the *hisT* mutation has no role in establishing the context effects (second hypothesis above); rather, the presence of *hisT* reduces (more than 10-fold) the activity of the suppressor at both sites. The *hisT* mutation prevents phenotypic suppression of *hisD6404* because *supE* is initially very inefficient at this site; the reduction in suppression caused by *hisT* reduces the amount of suppressed protein to a level below the threshold required for growth. The context change improves suppressor efficiency and, therefore, can be detected as a His⁺ revertant in *hisT*⁻ strains.

Context effects are tRNA specific

Context effects on tRNA efficiency involve aspects of tRNA structure other than simply the anticodon, because different tRNAs with the same anticodon are affected in different ways. Data on the effect of the *hisD6404* context change on the efficiency of various nonsense suppressors are presented in Table 5. A series of strains was constructed which carried *hisD6404* or *hisD6404-C1* and one of a series of amber suppressors. Each strain was assayed for its *hisD* enzyme level; enzymatic activity was taken as a measure of the translation efficiency at the amber site. As Table 5 shows, the context alteration of *hisD6404-C1* does not affect the efficiency of ochre suppressor *supG*; the change increases the efficiencies of amber suppressors *supE* and *supF*, but decreases the efficiency of amber suppressor *supD*.

For two reasons we conclude that the tRNA specificity of context effects is not due to qualitative differences in the suppressed proteins. First, the suppressed site is located in a non-essential part of the *hisD* protein. Second, the same effects of context on various suppressors are seen when polarity relief is used instead of *hisD* activity as a measure of suppressor efficiency. This was shown by assaying the level of *hisB* enzyme (Table 6). The *hisB* gene is located promoter-distal to *hisD* and is subject to polarity effects of the *hisD6404* mutation. While the magnitude of the context effect is reduced in these results, the general nature of the results agrees closely with the *hisD* assays in Table 5.

The results in Tables 5 and 6 provide further information on the nature of context effects. It has been proposed that the context effect on suppression can be explained by assuming

Table 3 mRNA sequence of the context mutant

Wild-type sequence	CAG	CAG	CGU
		↓	
<i>hisD6404</i> (amber)	CAG	UAG	CGU
			↓
<i>hisD6404-C1</i> (context mutant)	CAG	UAG	AGU

that the efficiency of peptide termination at a nonsense codon varies depending on the site of that codon^{1,2}. Our results argue against this explanation. If the context mutation in *hisD6404-C1* generates a less efficient termination signal, then the change should cause an increase in the efficiency of all suppressors at that site. This is clearly not the case for suppressors *supG* and *supD*. The tRNA specificity of the context effects is important to the possible implications of these effects for coding in general.

Discussion

We have shown that the efficiency of the amber suppressor *supE* in translating the UAG codon can vary over one order of magnitude depending on the nucleotide adjacent to the 3' side of the codon. The result demonstrates the role of codon context in determining suppression efficiency. In the mutant analysed, the suppressor inserts the same amino acid (glutamine) present in the wild-type sequence, and therefore measurements of enzyme activity directly reflect the actual efficiency of the *supE* tRNA in translation. Other authors have also shown that some site-specific differences in suppressor efficiencies are not due to protein differences¹⁻⁷. Several models have been proposed to explain these effects; one proposes that context effects result from variation in efficiency of termination at the nonsense site^{1,2}. The tRNA specificity data presented here argue strongly against that. Although the detailed explanation for context effects is unclear, two alternative hypotheses are consistent with our data.

According to the first, the nucleotide adjacent to the 3' side of the codon may influence the rate of initial tRNA interaction with the codon. Basically, the increase in the affinity of the *supE* tRNA anticodon for the UAG codon caused by the presence of an adenine residue (instead of a cytosine residue) at the position adjacent to the 3' side of the UAG, can be explained in two ways: (1) it may permit formation of a fourth base pair (A-U) involving the invariant U residue adjacent to the 5' side of the tRNA anticodon; or (2) it may increase the stacking energy of the codon-anticodon interaction. A uracil residue is present in the position adjacent to the 5' side of the anticodon in all the known tRNA species. The possibility that this nucleotide could base-pair with an A residue adjacent to the 3' end of the codon has been proposed to explain data analogous to ours; Tanaguchi and Weissmann showed that the binding efficiency of fMet-tRNA for the initiation codon, AUG, of the Qβ coat cistron is increased more than threefold when the G residue adjacent to the 3' side of the AUG in the wild-type sequence, is replaced by an A residue⁸. The hypothesis that such an additional base-pair is compatible with tRNA structure has been proposed also by Uhlenbeck *et al.*²⁷, after finding that the molar association constant (in absence of ribosomes) between fMet-tRNA and the tetranucleotide AUGA is more than 10-fold higher than that with AUGC, AUGG, AUGU, or that with the trinucleotide AUG. On the other hand, it is hard to explain the fact that other suppressor tRNAs (all carrying the U residue in the appropriate position) do not show an increase in efficiency at a codon followed by A (Table 5). Therefore, one should consider the alternative possibility that the nucleotide adjacent to the 3' end of the codon has a role in contributing to the stacking energy of the codon-anticodon interaction. This possibility is suggested by Grosjean *et al.*²⁸, who have shown the influence of the nucleotide adjacent to the 3' side of the anticodon on the stacking energy of anticodon-anticodon complexes. The behaviour of suppressors other than *supE*

could be explained by assuming that the effect of the nucleotide adjacent to the 3' end of the codon depends on particular features of the anticodon loop.

An alternative hypothesis to explain the context effects considers that the base change at the 3' side of the UAG codon generates a new neighbouring codon; the arginine codon, CGU, is replaced by the serine codon AGU. The efficiency of each translation step could depend on optimal 'fitting' of tRNA pairs on the ribosome. This hypothesis implies that tRNA-tRNA interactions occur when these molecules are occupying the A and P sites of the ribosome during translocation. Such interactions could affect the efficiency of the peptide elongation step. According to this hypothesis, *supE* tRNA is able to transfer the growing peptide chain more efficiently to the Ser-tRNA_{AGU} than to the Arg-tRNA_{CGU}.

Although the possibility of tRNA-tRNA interaction on the ribosome is speculative, several bits of genetic data support the notion. The best characterized frameshift suppressors are mutations which alter the structure of a tRNA such that a four-base code word is read, and the translational reading frame is shifted²⁹⁻³¹. This indicates that a given tRNA can dictate the distance that the ribosome moves along the message in the translocation step of protein synthesis. This process is easily understood if an incoming tRNA interacts with the incumbent tRNA in order to determine the position of the next code word. The frameshift suppressor tRNA, by occupying a four-base codon forces a codon in the +1 frame to be read by tRNA in the next translation step. The ribosome slavishly translocates this new, improperly positioned tRNA to the peptidyl site in the translocation step.

A recently characterized frameshift suppressor, *sufJ*, further supports the idea that tRNA-tRNA interactions dictate reading frame. The *sufJ* suppressor reads any four-base code word whose first three bases are ACC (our work in preparation). The fact that any base can occupy the fourth position suggests that this fourth base is not actually read.

Table 4 Effect of context on suppression efficiency

Relevant genetic background	HisD activity resulting from suppression of indicated site		
	(wild type) -CAGC-	(<i>hisD6404</i>) -UAGC-	(<i>hisD6404-C1</i>) -UAGA-
Wild type	107.3 (TR5830)	<0.2 (TT4427)	<0.2 (TT4428)
<i>supE</i>	100.0 (TT4424)	2.1 (TT4241)	21.2 (TT4268)
<i>supE hisT</i>	93.3 (TT4425)	<0.2 (TT4242)	1.0 (TT4271)

A set of otherwise isogenic strains was built carrying either *hisD*⁺, or *hisD6404*, or *hisD6404-C1* (context alteration) in addition to combinations of mutant and wild-type alleles of *supE* and *hisT*. The number and the full genotype of these strains is as follows:

TR5830 (*his01242 leu-414_{am}*)
 TR4424 (*his01242 leu-414_{am}supE20 zej-636::Tn5**)
 TT4425 (*his01242 leu-414_{am}supE20 zej-636::Tn5* hisT1504*)
 TT4427 (*his01242 hisD6404_{am}leu-414_{am}zej-636::Tn5**
zeb-618::Tn10†)
 TT4241 (*his01242 hisD6404_{am}leu-414_{am}supE20 zej-636::Tn5**)
 TT4242 (*his01242 hisD6404_{am}leu-414_{am}supE20 zej-636::Tn5**
hisT1504)
 TT4428 (*his01242 hisD6404_{am}-C1 leu-414_{am}zej-636::Tn5**
zeb-618::Tn10†)
 TT4268 (*his01242 hisD6404_{am}-C1 leu-414_{am}supE20 zej-636::Tn5**)
 TT4271 (*his01242 hisD6404_{am}-C1 leu-414_{am}supE20 zej-636::Tn5**
hisT1504)

Radiological assays of the histidinol dehydrogenase enzymatic activity were carried out according to Ciesla *et al.*²⁵. The value of the activity in strain TT4424 (*hisD*⁺ *supE*) was taken as 100; all other activities are expressed relative to this value. The results are the average of three determinations.

**zej-636::Tn5* designates a transposable kanamycin-resistance element co-transducible with the *hisT* locus.

†*zeb-618::Tn10* designates a transposable tetracycline-resistance element inserted at 41 min of the *Salmonella typhimurium* genetic map.

Table 5 Effect of codon context on several suppressors

Suppressor genotype	HisD enzyme activity resulting from suppression of indicated site	
	(hisD6404) -UAGC-	(hisD6404-C1) -UAGA-
sup ⁺	<0.2 (TT4427)	<0.2 (TT4428)
supG (lys)	0.8 (TT4286)	1.0 (TT4287)
supF (tyr)	3.4 (TT4278)	7.7 (TT4280)
supD (ser)	16.6 (TT4274)	6.7 (TT4276)
supE (gln)	2.0 (TT4241)	19.8 (TT4268)

Isogenic strains were built carrying either *hisD6404* or *hisD6404-C1* and the nonsense suppressors listed. The genotype of these strains is as follows:

TT4286:his01242 *hisD6404*_{am} *leu-414*_{am} *supG50* *zej-636::Tn5*
 TT4287:his01242 *hisD6404*_{am}-C1 *leu-414*_{am} *supG50* *zej-636::Tn5*
 TT4278:his01242 *hisD6404*_{am} *leu-414*_{am} *supF30* *zej-636::Tn5*
 TT4280:his01242 *hisD6404*_{am}-C1 *leu-414*_{am} *supF30* *zej-636::Tn5*
 TT4274:his01242 *hisD6404*_{am} *leu-414*_{am} *supD10* *zej-636::Tn5*
 TT4276:his01242 *hisD6404*_{am}-C1 *leu-414*_{am} *supD10* *zej-636::Tn5*

The genotypes of *sup*⁺ and *supE* containing strains are in the legend to Table 4. The enzymatic activity of the histidinol dehydrogenase was assayed as described in the legend to Table 4. Enzyme levels presented are relative to the level measured in strain TR5830 (*hisD*⁺ *sup*⁺). Results are the average of three determinations.

Table 6 Effect of codon context on the suppressor ability to relieve polarity

Suppressor genotype	HisB enzyme activity resulting from relief of polarity at the indicated HisD site	
	(hisD6404) -UAGC-	(hisD6404-C1) -UAGA-
supF (tyr)	5.6 (TT4278)	9.4 (TT4280)
supD (ser)	10.6 (TT4274)	5.9 (TT4276)
supE (gln)	0.9 (TT4241)	6.0 (TT4268)

HisB enzyme level was determined according to Martin *et al.*²⁶. The activity measured in strain TR5830 (*hisD*⁺ *sup*⁺) is 17.1. All the values are corrected by subtraction of the *hisB* enzyme level in strains TT4427 (*hisD6404* *sup*^{WT}) or TT4428 (*hisD6404-C1* *sup*^{WT}). Strains carrying ochre suppressor *supG* gave *hisB* activity values below the detection level. Strain genotypes are in the legends to Tables 4 and 5.

Instead, the suppressor tRNA may read ACC and sterically prevent any tRNA from occupying the codon immediately adjacent to ACC. Thus the next tRNA must read the next unhindered triplet (out of phase).

If our observations on context effects for nonsense suppression apply to decoding in general, one is led to some interesting conjectures. If the efficiency of reading any particular codon can vary over a 10-fold range, depending on codon context, it is clear that these effects could have enormous influence on the rate of protein synthesis in particular regions. The phenomenon could be used by the cell as an additional regulatory device. According to translation modulation theories, translation rates can be set by the use of codons whose tRNAs are present at a low level in the cell³²⁻³³. As Stent pointed out³³, modulation of translation would more specifically be achieved by using modulating sequences longer than the codon. Invocation of context effects seems, therefore, especially appropriate. A particular rare codon may or may not have modulating function depending on the reading context in which it is located.

The possibility that context affects translation suggests a role for the minor isoaccepting tRNA species whose presence has been reported in many organisms. Changes in the abundance of these minor species have been observed during changes in growth conditions³⁴, development³⁵⁻³⁷ and neoplasia³⁸. It seems possible that these minor tRNA species might be able to read their particular codon when it occurs in a context which is poorly read by the isocoding major tRNA species. Shifts in these minor tRNAs could vary the overall

rate of translation or could optimize production of proteins synthesized in large amounts in particular conditions.

Three examples may support these speculations. The first concerns the finding by Zilberstein *et al.*³⁹ that extracts of interferon-treated cells cannot completely translate haemoglobin mRNA unless a purified minor Leu-tRNA species is added. This minor Leu-tRNA could not be replaced by the major isocoding (CUG) tRNA species. The second case concerns tRNA genes carried by bacteriophage T4 (and other viruses). Guthrie and McClain have shown that one of the T4 tRNAs is essential for phage growth on a natural isolate of *Escherichia coli*⁴⁰. The essential tRNA is a minor Ile-tRNA species recognizing the codon AUA. A tRNA species with the same codon specificity is, of course, already present in the infected host. It seems possible that the viral tRNAs permit translation of T4 messages in which the context of some codons permits only inefficient reading by the tRNA complement of some natural hosts. The third example is the *Bombix mori* posterior silk gland in which a novel Ala-tRNA species appears while huge amounts of silk protein are being produced^{37,41}. One can speculate that to produce a large quantity of a particular protein, it may be necessary to eliminate even minor problems of context inherent in translation of message for that protein. The new silk gland Ala-tRNA may serve such a purpose. These three examples can all be interpreted as extreme cases in which there are restrictions of codon context that can be overcome by particular tRNA species. In summary, our data on the tRNA specificity of context effects suggest that any given codon may be read preferentially by one or another member of an isocoding tRNA family, depending on the context in which that codon appears.

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Analysis of regulation of *Klebsiella pneumoniae* nitrogen fixation (*nif*) gene cluster with gene fusions

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Gene fusions in which the lac genes are under the control of each promoter in the Klebsiella pneumoniae nitrogen fixation (nif) gene cluster have been constructed. These fusions have been used to examine positive control of the cluster and the response of individual genes to repression by ammonia and oxygen. De-repression of nif transcriptional units is coordinate and molybdate is required for maximal expression of the structural gene operon, which is autogenously regulated.

THE process of biological nitrogen fixation, the conversion of nitrogen to ammonia, is rate-limiting in many agricultural areas. Nitrogenase, the enzyme complex which catalyses the reduction of dinitrogen, is comprised of two redox proteins both of which contain iron-sulphur centres and one of which also contains molybdenum¹. These component proteins are irreversibly inactivated by oxygen. A cofactor (FeMo-co) containing Mo, Fe and S, presumed to carry the active site of nitrogenase, can be separated from the Mo-Fe protein². Both a reductant and ATP are required for enzyme activity³; approximately 15 mol of ATP are consumed per mol dinitrogen reduced. In view of this high energy requirement and the extreme oxygen sensitivity of the component proteins it is not surprising that nitrogenase synthesis is tightly controlled.

The nitrogen fixation (*nif*) gene cluster of *Klebsiella pneumoniae* consists of at least 15 genes organized into 5 polycistronic and 3 monocistronic transcriptional units (Fig. 1)⁴⁻⁷. The structural genes *nifK* and *nifD* code for the β and α subunits, respectively, of nitrogenase Kp1 (Mo-Fe protein) and *nifH* codes for Kp2 (Fe protein) of nitrogenase. The *nifB*, *nifN* and *nifE* gene products are involved in the synthesis or processing of the iron-molybdenum cofactor, and

the *nifM* gene product is required for Kp2 activity⁸. *NifF* and *nifJ* are involved in electron transport to nitrogenase⁹. The phenotype of *nifA* mutants suggests that *nifA* gene product is a positive activator of *nif* transcription^{8,10}. The functions of the remaining five gene products are unknown.

Regulatory studies have so far indicated that the nitrogenase structural genes are subject to repression by ammonia and by oxygen¹¹ and there is some evidence that molybdate is involved in the regulation of nitrogenase¹². In addition, glutamine synthetase, the product of the *glnA* gene, has been implicated as a general positive effector of enzyme systems which, including nitrogenase, are involved in the assimilation of 'poor' nitrogen sources¹³. There are, however, serious obstacles to studies on regulation of the individual transcriptional units in the *nif* gene cluster, because most of the gene products have not been conclusively identified and there is no simple assay for any of the individual gene products. To overcome these difficulties we have used the recently developed technique of Casadaban and Cohen¹⁴ to construct gene fusions in which the *Escherichia coli lac* genes are fused to a promoter of interest. Such gene fusions have enabled us to monitor the response of each

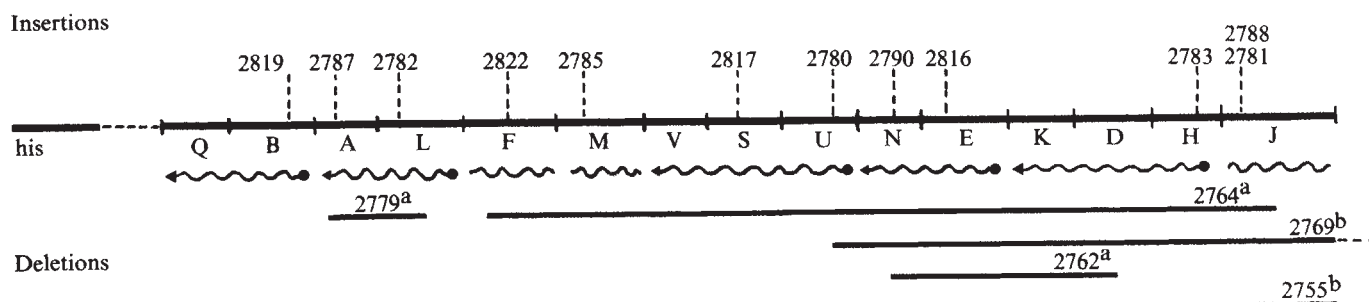


Fig. 1 Map of the *K. pneumoniae nif* gene cluster showing the location of insertions and deletions induced by phage Mud(Aplac). The wavy lines indicate the eight transcriptional units and the arrows show the direction of transcription where known. The map is not drawn to scale. Transposition of Mud(Aplac) into the conjugative plasmid pMF100, which carries the *his-nif* region of the *K. pneumoniae* genome was induced either by induction of an *E. coli* Mud(Aplac) lysogen, UNF511 (*his trp lys lacA str*) carrying pMF100 at 37 °C or by infection of *E. coli* strain JC3272 carrying pMF149 (a Mu-sensitive derivative of pMF100) with a mixed lysate of Muets and Mud(Aplac) at 30 °C. Insertion mutations in the plasmid were selected on the basis of co-transfer of *his* and ampicillin resistance in crosses carried out at 30 °C with an *E. coli* Muets lysogen, UNF514 (*his trp lacA recA56 spe*). In both cases the transposition frequency of Mud(Aplac) was approximately 10^{-3} with respect to plasmid transfer. *nif-lac* fusions were obtained by screening for colonies which failed to grow anaerobically on nitrogen-free (NFD²⁰) agar, but could form blue colonies on NFD plates containing serine (100 μ g ml⁻¹) and the indicator dye 5-bromo-4-chloro- β -D-galactoside (40 μ g ml⁻¹). Mud(Aplac) insertions were mapped against a set of *K. pneumoniae nif* point and deletion mutations described in ref. 7. Allele numbers above the map are independent insertions in which β -galactosidase is under the control of a *nif* promoter. Black lines beneath the map represent Mud(Aplac)-induced deletions in which (a) β -galactosidase is repressed by ammonia (under *nif* control) or (b) β -galactosidase is constitutive (under the control of a promoter outside *nif*).

Table 1 β -Galactosidase activities of *nif-lac* fusion strains in various genetic backgrounds

Fusion allele	β -Galactosidase units in:									
	UNF619 <i>nif</i> ⁺			UNF686 Δ (<i>his-nif</i>)			UNF1716 Δ (<i>his-nif</i>) <i>glnA201</i>	UNF1721 Δ (<i>his-nif</i>) <i>gln-205</i>	UNF686 Δ (<i>his-nif</i>) <i>nifA2013::Tn10*</i>	
	-NH ₄ ⁺ + Mo	-NH ₄ ⁺ - Mo	+NH ₄ ⁺	-NH ₄ ⁺ + Mo	-NH ₄ ⁺ - Mo	+NH ₄ ⁺	-NH ₄ ⁺	-NH ₄ ⁺	-NH ₄ ⁺	
<i>nif</i> B2819 :: Mud(Aplac)	400	250	9	440	250	6	24	9		
<i>nif</i> A2787 :: Mud(Aplac)	790	—	24	940	—	14	13	8		
<i>nif</i> A2779 :: Mud(Aplac)	710	600	20	1,040	600	17	16	7	—	
<i>nif</i> L2782 :: Mud(Aplac)	730	600	25	890	700	23	13	9	—	
<i>nif</i> F2822 :: Mud(Aplac)	700	400	16	700	400	15	56	35	—	
<i>nif</i> M2785 :: Mud(Aplac)	900	900	22	520	500	24	13	8	25	
<i>nif</i> S2817 :: Mud(Aplac)	220	—	18	120	—	11	11	19	10	
<i>nif</i> U2780 :: Mud(Aplac)	900	550	15	680	800	14	11	7		
<i>nif</i> N2790 :: Mud(Aplac)	950	500	23	580	500	14	9	9	19	
<i>nif</i> E2816 :: Mud(Aplac)	610	—	10	660	—	7	19	9	—	
<i>nif</i> H2783 :: Mud(Aplac)	3,500	500	7	500	500	11	9	8	9	
<i>nif</i> J2781 :: Mud(Aplac)	1,070	1,000	60	1,050	1,000	111	222	120	263	

Derivatives of pMF100 carrying the Mud(Aplac) insertions with or without a Tn10 insertion in *nifA* were introduced into various *K. pneumoniae* strains. All strains are *lacA* MuhP1 lysogens derived from KP5022 (*nif*⁺ *hisD2* *hsdR1*) or UNF1522 (Δ (*his-nif*) 2633 *hsdR1* str). UNF619 and UNF686 are also *recA*. Cultures were grown anaerobically in bijoux bottles in nitrogen-free medium (NFDm)²⁰, containing 2 mg ml⁻¹ (NH₄)₂SO₄ (+NH₄⁺) or 100 µg ml⁻¹ serine in the presence (-NH₄⁺, +Mo) or absence (-NH₄⁺, -Mo) of molybdate (100 µM). In the latter case, removal of traces of molybdenum was achieved by extracting both glucose and phosphate with 8-hydroxyquinoline and chloroform²¹, and by washing the glassware in 50% nitric acid (v/v). Cells were collected, resuspended in ice-cold saline phosphate buffer containing 100 µg ml⁻¹ chloramphenicol and assayed for β -galactosidase using the procedure and units of activity described by Miller²². Dashes indicate that the appropriate value was not determined.

*The *nifA2013::Tn10* (ref. 7) insertion was transduced onto the plasmids with phage PICmclr100 by selecting for tetracycline resistance and screening for cotransfer of both tetracycline and ampicillin resistance.

transcriptional unit to various effectors and to determine the kinetics of de-repression of individual operons.

Construction of *nif-lac* fusions

We used phage Mud(Aplac)¹⁴, a defective derivative of Mu which retains Mu transposition functions, carries part of the transposon Tn3 encoding ampicillin resistance (Ap) (which cannot itself transpose) and has the *lac* structural genes inserted in the phage such that when the phage inserts into a gene in the appropriate orientation, transcription proceeds through the S end of Mu into *lacZ*.

We have selected a large number of *nif-lac* fusions after transposition of Mud(Aplac) into the Nif⁺ conjugative plasmid pMF100. Around 20% of Mud(Aplac) insertions resulted in a Nif⁻ phenotype, although the majority of insertion events (261 out of 315 mutants obtained from several independent crosses) produced deletions which mapped to one side of the prophage. *lac* was expressed in some of these deletions and two classes were observed: (1) those with end points within *nif* in which *lac* was under the control of a *nif* promoter and was repressed by ammonia, and (2) those with more extensive deletions in which *lac* was fused to a promoter outside *nif*, resulting in the expression of β -galactosidase in the presence of ammonia (Fig. 1). All *nif-lac* fusions which did not carry adjacent deletions gave poor or no growth on lactose in the presence of ammonia. Precise mapping of these non-deleted strains has shown that the fusions are located in 11 out of 15 *nif* genes, with at least one fusion in each of the transcriptional units (Fig. 1).

Ammonium repression

Measurements of β -galactosidase activity in *K. pneumoniae* strains carrying various fusions suggest that all *nif* promoters respond to NH₄⁺ repression, although the extent of repression varies among different fusions (Table 1). These observations confirm and extend previous data on ammonia repression of some of the *nif* transcriptional units as measured by RNA-DNA hybridization experiments¹⁵ or by detection of *nif*-specific polypeptides⁸.

Positive control

The *nifA* gene product has been suggested to act as a specific positive control factor for *nif* transcription^{8,10}. We have therefore investigated the role of *nifA* in the regulation of the *nif* genes by constructing *nifA*, *nif::lac* double mutants. Transposon Tn10 was inserted into the *nifA* gene, thus

inactivating its function, and in all fusions tested, β -galactosidase activity was decreased considerably in the absence of an active *nifA* product (Table 1). Introduction of plasmids carrying a *nifA* mutation and a *nif-lac* fusion into a *nifA*⁺ background restored β -galactosidase activity (data not shown). However, the *nifA* gene product is not apparently required for transcription from the *nifL* promoter, as significant levels of β -galactosidase were observed in the *nifL* and *nifA* fusions and in the fusion *nif2779::Mud(Aplac)*, which is deleted for most of *nifA* and *nifL*.

The properties of various mutations affecting the synthesis or activity of glutamine synthetase suggest that either the enzyme itself or a product of one or more regulatory genes, including *glnF* and *glnG* (*glnR*), acts as a general positive effector for various operons involved in nitrogen metabolism^{16,17}. The response of *nif-lac* fusions to two *gln* mutations, *glnA201*, a mutation in the structural gene for glutamine synthetase, and *gln-205*, a *glnA*-linked regulatory mutation which prevents *nif* de-repression (G.E. and M.M. unpublished), is shown in Table 1. Transcription of all *nif* operons is affected by these mutations. This result does not allow us to distinguish whether the positive effector acts at only a single site within *nif* or whether all *nif* transcriptional units are subject to this control. Previous studies on *nif*-specific mutations which are independent of this regulation suggest that the effector may act at a single *nif* promoter and that the other transcripts are positively controlled by a *nif*-specific regulatory protein¹⁸. Our data are consistent with this model, as we have shown in Table 1 that transcription of the *nifLA* operon is dependent on a *gln* gene product.

The *nifJ* gene showed a unique response to various effectors. A high level of residual activity was observed in the *nifJ* fusion both in *glnA*, *gln-205* and *nifA::Tn10* backgrounds as well as in ammonia-grown cultures (Table 1). Similar results were obtained with an independent *nifJ* fusion, *nifJ2788::Mud(Aplac)*. It is possible that there is a less stringent requirement for *nifA* product at the *nifJ* promoter.

Regulation of the *nifHDK* operon

In the majority of cases, no significant difference was observed in the steady-state levels of β -galactosidase when merodiploid strains of genotype *nif*⁺/*nif::lac* were compared with haploid strains of genotype Δ *nif*/*nif::lac*. The exception was a *nifH::lac* fusion which showed a seven-fold increase in a *nif*⁺ compared with a total *nif* deletion background (Table 1), suggesting that this operon is autogenously regulated.

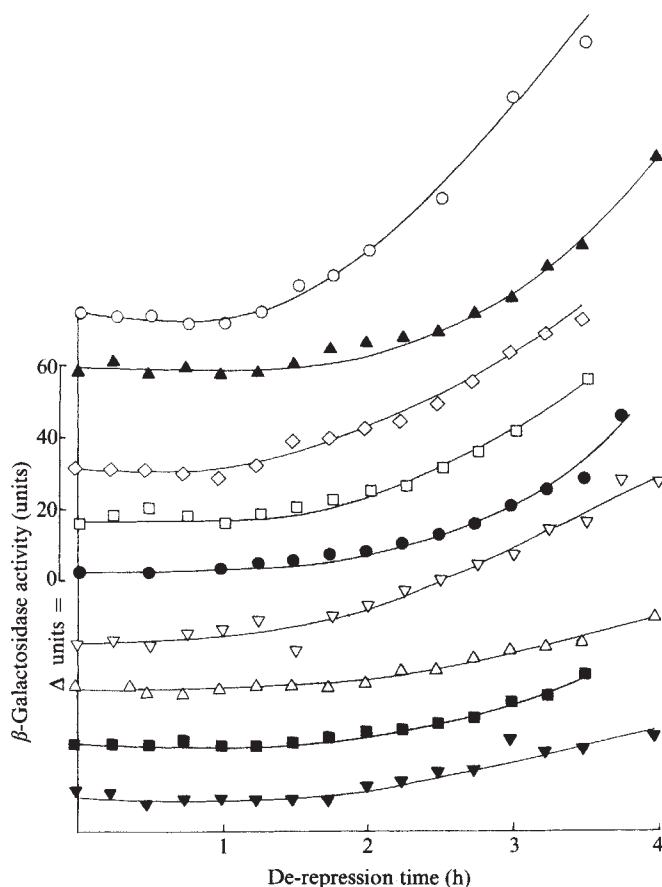


Fig. 2 Kinetics of de-repression of *nif-lac* fusions. Cultures were grown in NFD medium plus $(\text{NH}_4)_2\text{SO}_4$ (1.5 mg ml^{-1}) overnight at 28°C under N_2 , collected by centrifugation and resuspended in NFD medium at 25°C . Cultures were bubbled with N_2 and samples (0.2 ml) removed at the times indicated for estimation of β -galactosidase activity. For clarity the curves are displaced and the data on the ordinate plotted as the change in β -galactosidase activity (units) observed during de-repression. All strains were derivatives of UNF619 (*nif*⁺ *hisD2 hsdR1 lacA recA56 sr1300::Tn10* MuhP1) carrying *nif-lac* fusions in pMF100 (shown in Fig. 1). $\circ$, *nif* H2783::Mud(Aplac); $\blacktriangle$, *nif* N2790::Mud(Aplac); $\diamond$, *nif* M2785::Mud(Aplac); $\square$, *nif* J2781::Mud(Aplac); $\bullet$, *nif* F2822::Mud(Aplac); ∇ , *nif* U2780::Mud(Aplac); $\triangle$, *nif* B2819::Mud(Aplac); $\blacksquare$, *nif* L2782::Mud(Aplac); $\blacktriangledown$, *nif* A2787::Mud(Aplac).

Because the *nifH*::Mud(Aplac) insertion exerts a strong polar effect on *nifD* and *nifK*, it is possible that any of the products of the operon could affect the level of *nifH* expression. Table 2 shows the β -galactosidase activities of merodiploid strains carrying various mutations in *trans* to a *nifH*::*lac* fusion on the *K. pneumoniae* chromosome. (Levels of activity were generally lower than those with *nifH*::*lac* located on the plasmid (Table 1), suggesting a possible effect of plasmid copy number, and in these experiments there was a threefold increase in the diploid (*nifH*::*lac*/*nif*⁺) strain compared with the haploid (*nifH*::*lac*) level.) When the plasmid carried a Tn7 insertion in *nifK* or *nifD* or a deletion extending from *nifL* into *nifD*, β -galactosidase activities were 33–55% of the *nif*⁺ level. However, not all Tn7 insertions in *nifH* gave a significant decrease in β -galactosidase activity (Table 2); this may reflect differences in polarity exhibited by Tn7 insertions in *nifH*. Only three out of seven Tn7 insertions in *nifH* had reduced levels of activity, whereas two *nifD* and three *nifK* insertions exhibited less than 55% of the *nif*⁺ control (data not shown). These results suggest that the *nifK* and/or *nifD* gene products are involved in regulation of the operon. As none of the mutations tested can complement the *nifH*::*lac* insertion, nitrogenase activity *per se* is not required for stimulation of *nifH* transcription.

Involvement of molybdenum

Molybdenum is essential for nitrogenase activity, as it is a component of the iron-molybdenum cofactor of Kp1 protein. Hence, growth on molecular nitrogen is dependent on the presence of molybdate in the medium. As there is conflicting evidence concerning the involvement of molybdate in the regulation of nitrogenase synthesis in *K. pneumoniae*^{12,19}, we have re-examined this question using *nif-lac* fusion strains grown in the presence or absence of molybdate ($100 \mu\text{M}$). The absence of molybdate did not affect the β -galactosidase activity of ammonia-grown *nif-lac* fusion strains. None of the operons showed an absolute requirement for molybdate for de-repression and, in the haploid situation, β -galactosidase activities did not differ significantly in the presence or absence of molybdate (Table 1). Thus, molybdate has no regulatory function *per se*. In the diploid situation, only the *nif*⁺/*nifH*::*lac* strain responded to molybdate addition and *lac* expression was increased sevenfold (Table 1). These results show that both the presence of molybdate and product(s) of the *nifHDK* operon are required for maximal expression from the *nifH* promoter. It is therefore most likely that a molybdo-protein, presumably Kp1 protein, is involved in autogenous regulation of the operon. We have been unable to distinguish whether the presence of molybdate influences the stability of this protein or whether insertion of molybdenum into Kp1 is necessary for the formation of a regulatory active species.

Oxygen repression

Oxygen represses nitrogenase synthesis as well as inactivating the nitrogenase component proteins¹¹. Synthesis of β -galactosidase from all the *nif* promoters was inhibited in both *nif*⁺ and *nif* deletion backgrounds when NH_4^+ -grown cultures were resuspended in N-free medium and bubbled vigorously with air for 6 h. The degree of expression among different fusions varied from 3% (*nifH*) to 43% (*nifL*) of the activity obtained with control cultures sparged with nitrogen.

Additional experiments were carried out in an oxystat in which a defined and constant dissolved oxygen tension (D.O.T.) could be maintained. When a *nif*⁺ strain was grown in ammonia, resuspended in N-free medium and maintained at a D.O.T. of 0.5%, de-repression was inhibited and synthesis of nitrogenase polypeptides could not be detected in pulse-labelling experiments (S. Hill and C. Kennedy, unpublished observations). Synthesis of β -galactosidase from the *nifH* promoter was also repressed as expected in these conditions, but synthesis from the *nifL* promoter in both *nif*⁺ and *nif* deletion backgrounds was unaffected (Table 3). Thus, oxygen repression of the *nifHDK* operon is not mediated by inhibiting transcription at the *nifLA* promoter, at least at low oxygen tensions. At higher D.O.T.s (3% and 5%, Table 3) the *nifL*::*lac* fusion was apparently subject to oxygen repression as predicted from the bubbling experiments. The *nifHDK* operon is, therefore, far more sensitive to oxygen repression than the *nifLA* operon.

Table 2 Effect of mutations in *trans* on β -galactosidase synthesis from *nifH*::*lac*

Plasmid allele in <i>trans</i>	β -Galactosidase units	% <i>nif</i> ⁺
—	385 ± 35	30
<i>nif</i> ⁺	$1,269 \pm 107$	100
<i>nif</i> K2185::Tn7	481 ± 90	38
<i>nif</i> D2197::Tn7	530 ± 20	42
<i>nif</i> D2210::Tn7	410 ± 3	32
<i>nif</i> A(L-D)8758	618 ± 68	49
<i>nif</i> H2191::Tn7	$1,095 \pm 25$	87
<i>nif</i> H2212::Tn7	$1,137 \pm 67$	90
<i>nif</i> H2213::Tn7	533 ± 37	42

Derivatives of plasmid pRD1 carrying *nifA*(L-D)8758 or Tn7 insertions in *nifH*, *nifD* or *nifK* (ref. 7) were introduced into UNF725 (*nif* H2783::Mud(Aplac) *hsdR1 lacA recA56 sr1300::Tn10* MuhP1). Cultures were grown and assayed for β -galactosidase activity as in Table 1.

Table 3 Effect of oxygen on β -galactosidase activity in *nifL* and *nifH* fusions

Fusion allele on plasmid	Strain background	Cultures bubbled with air	Units of β -galactosidase activity under O ₂ as % of that under N ₂		
			Cultures maintained at a D.O.T. (% saturation) of:		
			0.5	3.0	5.0
<i>nifL</i> 2782	$\Delta(his-nif)$	32 $\pm$ 9	123 $\pm$ 10	68	54 $\pm$ 6
	<i>nif</i> ⁺	43 $\pm$ 17	116 $\pm$ 3	64	—
<i>nifH</i> 2783	$\Delta(his-nif)$	10 $\pm$ 3	32 $\pm$ 3	—	—
	<i>nif</i> ⁺	3 $\pm$ 1	15 $\pm$ 3	—	—

Cultures were grown in NFDM²⁰ plus excess NH₄⁺ overnight at 30 °C under N₂, collected by centrifugation and resuspended in NFDM at 30 °C. β -galactosidase activity was measured after vigorously bubbling 5 ml aliquots of culture with either air or N₂ for 6 h. Alternatively, β -galactosidase activity was determined after 4.5 h exposure either to the indicated D.O.T. in an oxystat supplied with air plus N₂, or to N₂. —, Not tested.

Kinetics of *nif* de-repression

Pulse-labelling experiments on cultures released from NH₄⁺ repression have shown that the *nifK*, *nifD* and *nifH* polypeptides are synthesized approximately 1 h before nitrogenase activity can be detected, indicating either that de-repression of *nif* operons is not coordinate or that there is a rate-limiting step in establishing a functional nitrogenase system¹¹. To examine this question more thoroughly, the onset and rate of de-repression for diploid (*nif*⁺/*nif*::*lac*) fusion strains have been determined following transfer from NH₄⁺ to N-free medium. The appearance of nitrogenase activity measured by acetylene reduction was reproducible in all strains tested, occurring 2–2.5 h after transfer to N-free medium. Control experiments in which rifampicin (100 μ g ml⁻¹) was added before de-repression, indicated that there was no significant increase or decrease in the residual level of β -galactosidase activity in the absence of *nif* transcription. The onset of β -galactosidase de-repression occurred at 1–1.5 h in all cases, but the rates of enzyme synthesis varied among the different fusions (Fig. 2). Although the levels of β -galactosidase activity in fusion strains do not necessarily represent the efficiencies of the various *nif* promoters, the de-repression curve for *nifH* agrees well with pulse-labelling data on the rate of nitrogenase synthesis during de-repression¹¹, and the relative amounts of β -galactosidase synthesized from the other promoters generally correlate with the relative rates of synthesis of detectable *nif*-specific polypeptides observed on polyacrylamide gels. Note that synthesis of β -galactosidase from the *nifA*, *nifL* and *nifB* promoters occurred initially at a slower rate than with the other fusions. The slow de-repression of *nifB* may account for the late appearance of acetylene-reducing activity. Consistent with this hypothesis, nitrogenase activity of crude extracts prepared from cultures in the 'early' stages of de-repression is stimulated by addition of purified FeMo-co (R.R.E., unpublished results).

To examine regulation of the *nifHDK* operon in more detail, we also compared the de-repression kinetics of *nifH* diploid and haploid strains grown in the presence and absence of molybdate. Three typical curves are shown in Fig. 3: *a*, The *nif*⁺/*nifH*::*lac* diploid fusion in the presence of molybdate showed a linear increase in β -galactosidase activity from the onset of de-repression for at least 7 h after transfer from NH₄⁺ to N-free medium. *b*, The *nifH*::*lac* haploid strain, grown either with or without molybdate, initiated de-repression of β -galactosidase as in *a* but subsequently showed a cut-off in synthesis after 3.5–4 h in N-free medium. The *nif*⁺/*nifH*::*lac* diploid strain in the absence of molybdate also showed a cut-off similar to that of the haploid strain. (For simplicity only the curve for the haploid *nifH*::*lac* fusion in the presence of molybdate is shown in Fig. 3.) *c*, A *nifH*::*lac*, *nifA*::Tn10 double mutant showed no increase in β -galactosidase activity in the presence of molybdate, indicating that the *nifA* product is necessary for the onset of de-repression.

Thus, de-repression of the operon is clearly initiated in the absence of molybdate or in the haploid strain lacking the *nifH*, *nifD* and *nifK* products. The cut-off indicates there is an insufficient level of a regulatory molecule to maintain transcription from the *nifH* promoter in these conditions.

Discussion

Our data suggest that all the operons in the *nif* gene cluster are subject to positive control, which is ultimately mediated either by glutamine synthetase itself or by a gene product involved in glutamine synthetase regulation. We assume that this positive effector activates transcription at the *nifLA* promoter and that the *nifA* product specifically promotes transcription of other *nif* operons. Alternatively, it is conceivable that the positive effector involved in glutamine synthetase regulation is required in addition to the *nifA* product at these promoters. We have also shown that the *nifHDK* operon is subject to autogenous regulation and that the availability of molybdate and at least the *nifK* and *nifD* gene products are required for maximal expression from the operon promoter. This conclusion is also consistent with the finding that the level of Kp2 protein is low in strains carrying insertion mutations in *nifK* and *nifD*^{4,8}. In wild-type nitrogen-fixing bacteria, autogenous regulation of the *nifHDK* operon might provide a useful mechanism for preventing synthesis of inactive nitrogenase.

All transcriptional units in the *nif* gene cluster are repressed by ammonia and as there is no evidence for a *nif*-specific repressor which responds to ammonia, it is possible that this repression is mediated through the level of a *gln* gene product. The latter control system is probably not involved in oxygen repression because mutants which produce nitrogenase in the

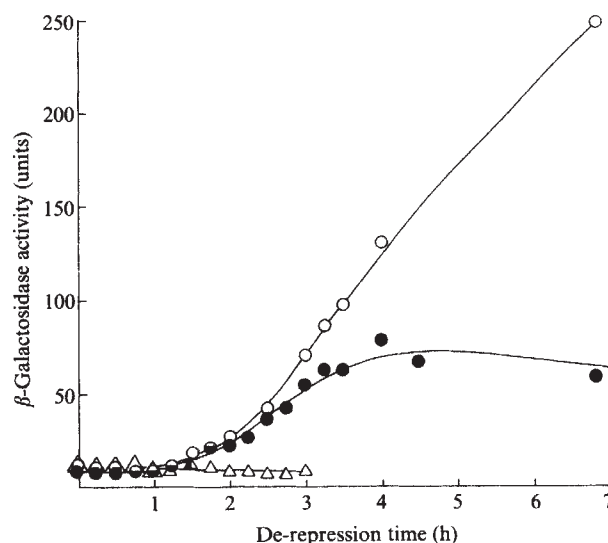


Fig. 3 Effect of strain background on de-repression kinetics of *nifH*::*lac*. Cultures were grown and assayed for β -galactosidase activity as in Fig. 2. Strains were derivatives of UNF619 and UNF686 described in Table 1 legend. *a*, *nif*⁺/*nifH*2783::Mud(Aplac) (○); *b*, $\Delta(his-nif)/nifH2783::Mud(Aplac)$ (●); *c*, $\Delta(his-nif)/nifH2783::Mud(Aplac)$, *nifA*2013::Tn10 (△). When grown in the absence of molybdate both diploid (*nif*⁺/*nifH*::*lac*) and haploid (*nifH*::*lac*) strains gave similar curves to ●. For description of curves *a*–*c*, see text.

presence of ammonia remain subject to oxygen regulation¹¹. It is also unlikely that the products of the *nifHDK* operon are involved in oxygen repression because the level of β -galactosidase activity in a haploid polar *nifH::lac* fusion strain was lower under a D.O.T. of 0.5% than the control under nitrogen. It is possible that one of the other products of the *nif* gene cluster acts as an oxygen sensor and can mediate oxygen repression at a low D.O.T., whereas at higher oxygen concentrations there is a more general effect on the *nif* gene

cluster which may also extend to other oxygen-repressible systems in the cell.

The isolation of regulatory mutations in each of the *nif* transcriptional units should considerably enhance our knowledge of the regulation of this complex gene cluster.

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LETTERS

IUE observations of the UV spectrum of comet Bradfield

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Comet Bradfield (1979I), discovered shortly after perihelion, on Christmas day 1979, is characterized by a retrograde orbit of period ≈ 250 yr. This orbit was very favourable for observation by the IUE satellite¹, and the first observations, the preliminary results of which are reported here, were made on 10 and 11 January 1980. Previously, comprehensive vacuum UV cometary spectra were available only for Comet West (1976VI) from rocket observations^{2,3} and Comet Seargent (1978m) obtained with IUE⁴, hence three comets have now been observed in the wavelength region where most of the major constituents are detectable spectroscopically. The UV spectra of these comets are remarkably similar, despite large differences between them in the gas-to-dust ratio and the different heliocentric distances at which the observations were made, and although the statistical basis is still very small, the implication for cosmogony is that nearly all comets have the same primary composition and origin.

At the time of these observations the comet was 0.71 AU from the Sun, 0.61 AU from Earth, and had a total visual magnitude ≈ 4 . The visual appearance of the comet was diffuse with a weak central condensation and with only a slight trace of an ion tail. The comet could be tracked on its centre of

brightness by the fine error sensor of the IUE. Drift was found to be no more than $1-2 \text{ arc s}^{-1}$. All observations were made with the large ($10 \times 20 \text{ arc s}$) apertures of the spectrographs and the pointing accuracy was verified by the presence of the weak continuum near $2,900 \text{ \AA}$ in the centre of the slit.

The short wavelength spectrum is shown in a 2-h exposure in Fig. 1. The vertical scale has been reduced to average surface brightness per $\text{\AA}$ in the $10 \times 20 \text{ arc s}$ slit. As noted below, the use of the large slit permits a limited amount of spatial imaging on extended sources⁵ to a resolution of $\approx 6 \text{ arc s}$, but the spectra presented here represent the average over the entire slit. The dominant features in the spectrum other than H I Ly α which is highly over-exposed are O I $\lambda 1,302$, C I $\lambda 1,561$, 1,657 and 1,931 and Si $\lambda 1,813$, as in the spectrum of Comet West^{2,3}. The brightness in a given line or multiplet is obtained by integrating over the instrumental spectral band-pass which varies from $\approx 6 \text{ \AA}$ for a point source to $\approx 11 \text{ \AA}$ for a diffuse source uniformly filling the entire aperture. Table 1 gives the average brightness for these features. The Cu I $\lambda 1,335$ multiplet and CO fourth positive bands observed by Feldman and Brune², are present in the smoothed spectrum of Fig. 1 slightly above the noise at about the same level relative to the C I lines as in the spectrum of Comet West. It is surprising that the spectrum of Comet

Table 1 Average brightness of spectral features at 0.71 AU

Species	Wavelength ($\text{\AA}$)	Brightness (kR)
H I Ly α	1,216	160
O I	1,304	0.33
C I	1,561	0.12
	1,657	0.55
	1,931	0.05
Si I	1,807-1,826	0.26
CS (0,0)	2,576	1.40
(0,1)	2,663	0.15
CO ₂ ⁺	2,890	0.60
OH (1,0)	2,820	7.6
(0,0)	3,085	370
(1,1)	3,142	12
CO ⁺ (0,1) + (1,2)	2,321	0.39
(0,2) + (1,3)	2,436	0.18

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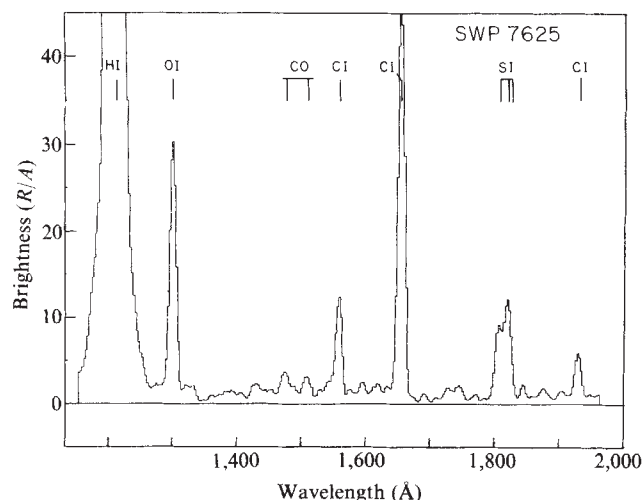


Fig. 1 Low dispersion, short wavelength spectrum of Comet Bradfield taken with the IUE spectrograph slit centred on the visible centre of brightness on 10 January 1980. The exposure time was 2 h.

Bradfield is so similar to that of Comet West considering the differences in appearance, heliocentric distance and gas production rate at the times of observation as well as the difference in projected slit area of the two instruments. This suggests that reactions among the neutral and ionized species in the inner coma may not play a significant part in altering the composition of the coma following photodestruction of the mother molecules evaporated from the cometary nucleus.

The average brightness of the H I Ly α emission, which is saturated in the spectrum of Fig. 1, is determined from a 60-s exposure and is found to be 160 kR which indicates an optically thick atomic hydrogen column along the line-of-sight to the centre of the coma. The O I multiplet at 1,302 Å is nearly optically thick as it is excited by resonance scattering of the solar λ 1,302 lines because the heliocentric velocity of the comet was 24 km s⁻¹. In previous observations of Comets Kohoutek and West² this velocity was $\approx$ 50 km s⁻¹ which shifted the cometary oxygen absorption outside the solar line profile and it was assumed that fluorescent pumping by solar H I Ly β radiation was the excitation source⁶.

A 2-h exposure of the long wavelength spectrum is shown in Fig. 2. Once again the dominant features are the CO⁺, CS, CO₂⁺, and OH bands identified previously³, but now the identification of CS is confirmed by the presence of the (0,1) band at 2,663 Å and the (1,0) band at 2,507 Å in addition to the saturated (0,0) band at 2,576 Å, all three of which are

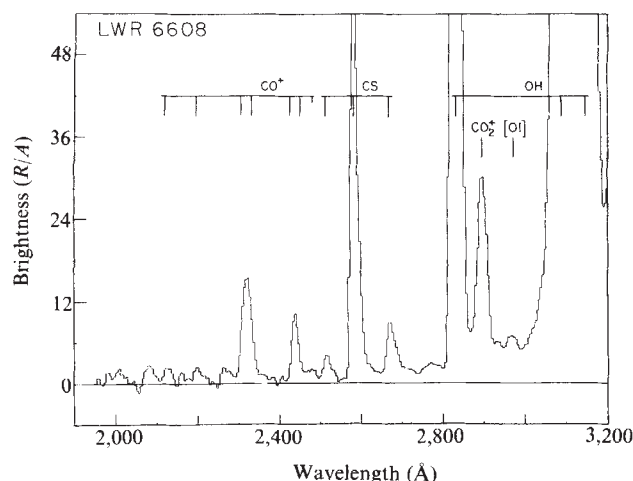


Fig. 2 Same as Fig. 1, but a 2-h exposure with the long wavelength spectrograph.

degraded towards the red. The three OH bands are also saturated but the average brightness of these bands can be found from shorter exposures. These are all listed in Table 1. The weak continuum observed near 2,750 and 2,950 Å suggests a very small dust-to-gas ratio for this comet, consistent with visual observations.

One peculiarity of this spectrum is the absence (or extreme weakness) of the (0,0) and (1,0) bands of the CO⁺ first negative system near 2,190 and 2,112 Å, respectively. Both of these bands are also absent or very weak in the IUE spectrum of Comet Sargent⁴. In the rocket spectra of Comet West^{2,3} the (0,0) band was the strongest CO⁺ feature. It does not seem likely that fluorescent pumping by solar radiation can explain this difference as the observed strong bands near 2,300 Å (0,1 and 1,2) and 2,420 Å (0,2 and 1,3) originate on the same $v''=0$ and 1 levels of the upper B² Σ^+ state of CO⁺ and in an optically thin ion coma the band intensities would be proportional to the Franck-Condon factors for the transitions. One possibility is that the CO⁺ in our field of view is optically thick along the line-of-sight in the $v''=0$ ground state and as a result (0,0) and (1,0) band photons are re-absorbed and pumped into transitions such as (0,1) and (1,2). The difference with the Comet West data can be explained by the difference in field of view, 5 $\times$ 35 arcmin for the experiment of Feldman and Brune² compared with 10 $\times$ 20 arcs for IUE, a factor of $\approx$ 3,200 in solid angle. Thus, most of the radiation observed by Feldman and Brune is from CO⁺ significantly further away from the nucleus where the CO⁺ is not optically thick along the line-of-sight, and the relative band intensities are close to the theoretically expected values. A consequence of this explanation is the inference that CO⁺ is produced in large abundance within 3,000 km of the nucleus. However, this hypothesis is not consistent with visual spectra obtained later in the month (January 30.0) which showed only very weak emission in the CO⁺ comet tail bands near 4,000 Å (C. McCracken, personal communication) and only a faint visual ion tail.

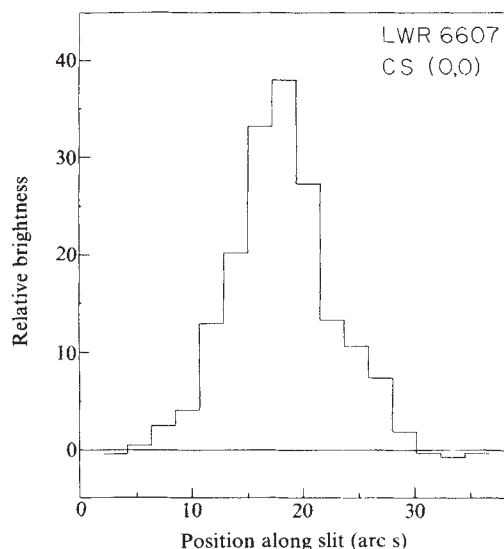


Fig. 3 Variation of the brightness of the CS (0,0) band at 2,576 Å across the spectrograph slit. At the instrumental resolution of 6 arcs, the CS seems to have a point-source origin.

Several very weak features were observed in both Fig. 1 and Fig. 2 and may, in fact, be real emission features such as additional CO fourth positive bands or the [O I] line at 2,972 Å. There is strong evidence that the [O I] 2,972 Å feature is real as it is also present above the dust continuum in the spectra of Comet West^{2,3}. This transition (¹S-³P), known to atmospheric spectroscopists as the 'trans-auroral' line of

oxygen, is produced at 5% of the rate of the 'auroral green line' at 5,577 Å ($^1\text{S}-^1\text{D}$). The green line has been proposed⁷ but not conclusively identified in cometary spectra due to the masking effect of the solar scattered continuum, the nearby (1,2) Swan band of C_2 and the presence of this line in the night airglow, but it is clear that oxygen in the ^1S state is produced in the coma. Preliminary calculations indicate that direct photodissociation of H_2O by solar H I Ly α radiation is sufficient to account for the observed surface brightness of the 2,972 Å feature.

The spatial variations of the UV emissions can be studied in two ways. For those emissions with short scale lengths there is an appreciable variation in brightness within the 10×20 arcs aperture of the spectrographs and the variation can be mapped with a spatial resolution of ≈ 6 arcs. Otherwise it is necessary to take several different exposures with the spectrograph slit offset by a known amount from the centre of visual brightness of the coma. An example of the former is the CS (0,0) band at 2,576 Å, for which the spatial variation in the large slit from a 30-min exposure is shown in Fig. 3. The horizontal scale is the linear distance from the centre of the coma appropriate to the Earth-comet distance at the time of observation. The sharp decrease in brightness away from the centre of the comet suggests a ρ^{-1} brightness dependence characteristic of a parent molecule density distribution which varies as R^{-2} close to the nucleus, where R is the distance from the centre of the comet and ρ is the distance from the centre projected onto the sky. It is thus very probable that CS exists as a parent molecule in the cometary ice or is the dissociation product of an extremely short-lived parent molecule.

The second technique, several exposures centred at different distances from the visible centre of brightness, is shown for the OH (0,0) band at 3,090 Å in Fig. 4. The three data points are compared with the predictions of a Haser model⁸ to give both the scale length (at 1 AU) and the OH production rate. However, the results are sensitive to the choice of the assumed H_2O outflow velocity and for our purpose we took extreme cases of 0.5 and 1.0 km s^{-1} . The other parameters needed⁹ are the H_2O lifetime, $8.2 \times 10^4 \text{ s}$, and the OH outflow velocity, 1.15 km s^{-1} . The two curves in Fig. 4, virtually indistinguishable from each other, correspond to values of $\tau_{\text{OH}} = 1.0 \times 10^5 \text{ s}$ and $5.0 \times 10^4 \text{ s}$ for the respective values of $v_{\text{H}_2\text{O}} = 0.5$ and 1.0 km s^{-1} . The corresponding values of the OH (and presumably H_2O) production rate at 0.71 AU are $Q_{\text{OH}} = 1 \times 10^{29} \text{ s}^{-1}$ and $2 \times 10^{29} \text{ s}^{-1}$, respectively. The range of deduced values of τ_{OH} (at 1 AU) is in accord with the theoretical value

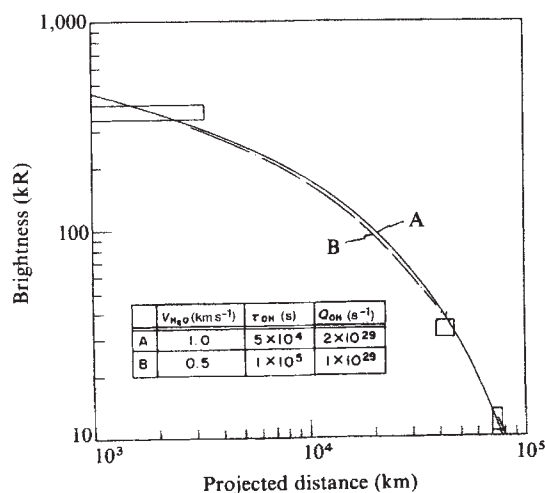


Fig. 4 Comparison of the OH (0,0) band brightness profile with a radial outflow model using the parameters defined in the insert. Data from three exposures are shown as rectangular boxes, the horizontal size being the projected length of the spectrograph slit on the comet and the vertical size the measurement uncertainty.

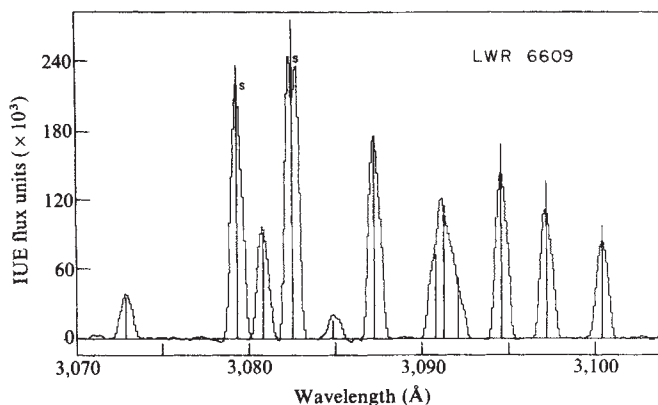


Fig. 5 High dispersion spectrum of the OH (0,0) band taken from a 15-min exposure. The wavelengths shown are vacuum wavelengths. The ordinate is in IUE flux units which are not corrected for the absolute spectral response of the instrument. The theoretical intensities have been multiplied by the approximate spectral response curve of the long-wavelength camera which varies steeply in this spectral region. The $P_1(1)$ line near 3,082 Å was saturated (S) in the observed spectrum and is off-scale in the theoretical calculation.

of $7.5 \times 10^4 \text{ s}$ given by Jackson¹⁰ for a heliocentric velocity of 24 km s^{-1} . According to Festou⁹, the lifetime derived by means of Haser's model is a lower limit to that obtained from a model which properly accounts for the spatial distribution of the OH radicals produced by photodissociation. The water production rate, reduced to 1 AU, of $0.5-1 \times 10^{29} \text{ s}^{-1}$, is considerably lower than that of several 'new' comets as given by Keller and Lillie¹¹, consistent with the identification of this comet as a long-period comet which has made numerous passes through the inner Solar System.

Assuming that CS is evaporated from the nucleus with the same outflow velocity as H_2O , it is possible to determine the relative production rate of these two molecules. A g-factor for the CS (0,0) band at 2,576 Å of 7×10^{-4} photons per s per molecule at 1 AU was adopted which leads to a value of the ratio $Q_{\text{CS}}/Q_{\text{H}_2\text{O}} \approx 5 \times 10^{-4}$. Thus, although CS is a prominent feature of the UV cometary spectrum it is clearly only a minor species in the comet.

The high resolution capability of the IUE spectrographs was used to study further the resonance fluorescence excitation of the OH (0,0) band, the strongest emission feature in the cometary spectrum. A 15-min, high-dispersion exposure is shown in Fig. 5, along with the predicted relative intensities as calculated for the heliocentric distance, r , and radial velocity, $\dot{r}$, at the time of observation¹². The excellent agreement between theory and observation over the velocity range $24-28 \text{ km s}^{-1}$ indicates that no process other than fluorescence is important in controlling the OH level populations. The dependence of the spectrum on $\dot{r}$ is strikingly illustrated by comparison of Fig. 5 with a similar, high-dispersion spectrum of this band from Comet Sargant (1,978m) (ref. 4), which differed in heliocentric radial velocity by $\sim 10 \text{ km s}^{-1}$. The large changes in the spectrum with radial velocity indicate that the fluorescence efficiency (g-factor) is also a function of radial velocity. The velocity dependent g-factors, needed to convert low resolution fluxes to column densities as above, were obtained from this high resolution analysis.

These new results are only the preliminary analysis of the data obtained at the first opportunity for IUE observations of Comet Bradfield. Observations were continued, at the rate of one a week corresponding to increments in heliocentric distance of $\sim 0.1 \text{ AU}$, through February, and should provide important information about the variation of the production rates of the UV active species with distance from the Sun. As the first comet to be so regularly observed in the vacuum UV, Comet Bradfield will greatly increase our understanding of the physics and chemistry of the cometary atmosphere.

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Note added in proof: Further analysis has disclosed that the features at 2,321 and 2,436 Å are incorrectly identified as CO⁺; rather they are a Mulliken band of C₂ and the second order HILy α image, respectively. The absence of CO⁺ bands in the UV is thus consistent with the absence of this species in the visible spectrum.

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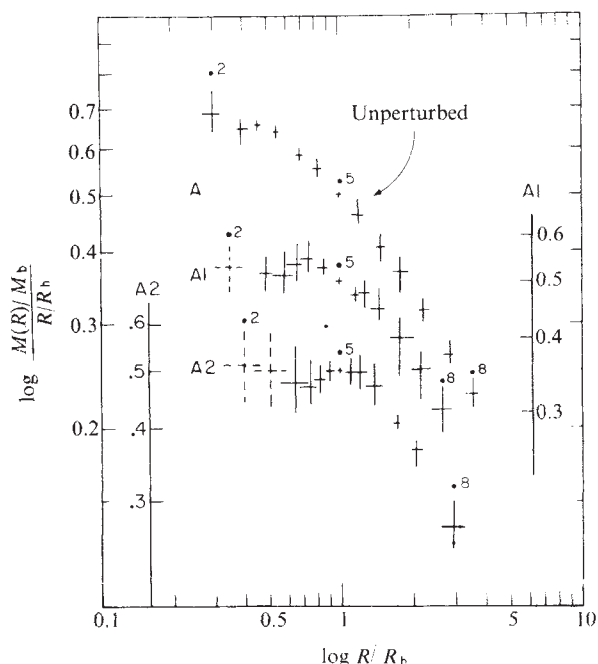


Fig. 1 Rotation curves of two tidally perturbed haloes, A1 and A2, are compared with the unperturbed one at the same final time. The mass and the radius are in fractional units relative to the mass, M_b , and the half-mass-radius, R_h , of the remnant bound system. The symbols correspond to given fractions of the bound mass, separated by 5%, and the error bars correspond to temporal deviations over three successive crossing-times. The curves are vertically shifted by a factor of 1/2 relative to each other. The parameters for the cases A1 and A2 are the following: perturber mass/halo mass = 2; relative velocity at closest approach = 1.05 the parabolic velocity; closest approach distance = $4.6 R_h$, $2.8 R_h$; fractional mass loss = 0.22, 0.33; final R_h /original R_h = 0.8, 0.74. Note that both the fractional and absolute flat regions become more extended.

Tidal effects on the mass profile of galactic haloes

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Most spiral galaxies are believed to be embedded in dark massive haloes^{1,2}. The main observational evidence for their presence is the rotation curves of edge-on disks at large radii measured both by optical^{3,23} and radio (21 cm)⁴ techniques: rotation velocities remain constant to distances of several tens of kiloparsecs, far beyond the main visible bodies of the galaxies. This suggests the existence of a dark halo whose mass, if spherical, varies linearly with distance R from the galactic centre and hence has a density profile which falls off as R^{-2} . The extended 'flat' shape of the haloes poses a problem for most theoretical hypotheses of galaxy formation because simulations of collapse⁵⁻⁸ and of violent mergers⁹ predict a spherical density profile which is rather Hubble-like ($\rho \propto R^{-3}$) or even steeper. The time scale for two-body relaxation, which can lead to a flatter, isothermal, density profile, is much larger than the Hubble time. If, as Gunn¹⁰ and Gott⁶ have suggested, secondary cosmological infall produces the $\rho \propto R^{-2}$ haloes, special initial conditions are required: (1) the central perturbation, which is the progenitor of the galaxy, needs to be initially embedded in a bound homogeneous background—an assumption which might not be fully justified for relatively isolated galaxies; and, (2) the infall must be such that there is no dissipation so that most of the mass should already be in the form of compact objects before halo formation. We suggest, as an alternative mechanism, tidal interactions between haloes, or possibly between their smaller building blocks, while in the hierarchical gravitational clustering process. In this process typical tidal encounters are slow, such that relative orbital velocities of the interacting systems are comparable to the internal velocities of the stars in each system. The two systems are slightly unbound.

Our suggestion is based on N -body simulations which we performed to study the tidal effects of such encounters on the mass-profile of spherical systems¹¹. On adopting the common view, that the dark material is not gaseous¹²⁻¹⁴ but is rather composed of condensed bodies (such as unevolved low-mass stars, dead remnants of old stars, and primordial black holes¹⁵), a dissipationless N -body system was assumed to represent properly a galactic halo. The numerical experiments

were based on the N -body code developed by Aarseth^{7,16,17}. It integrates the equations of motion of N softened particles, which interact through a potential

$$\phi_{ij} = -Gm_i m_j / [(r_i - r_j)^2 + \epsilon^2]^{3/2}. \quad (1)$$

The softening parameter, ϵ , is chosen to suppress the two-body relaxation effects that arise due to the small number of particles in our experiment.

In our experiments, a 250-body system was tidally perturbed by a point perturber of comparable mass. The encounter velocities were slightly above parabolic and the closest approach distances, p , were between $2.5 R_h$ and $5 R_h$, where R_h was the half-mass-radius. In such conditions, each 'shell' of stars expands, gradual stripping occurs outside $\sim R_h$ and up to one-third of the original mass may become unbound. As Fig. 1 shows, the effect on a system which initially has a decreasing $M(R)/R$ profile ($\rho \propto R^{-3}$) is to steepen the profile in the outer parts, or to produce, or extend, an inner region of constant $M(R)/R$, to encompass 50–70% of the bound mass. Hence, this mechanism, whose exact relaxation nature is not yet understood theoretically, produces systems which might show flat rotation curves over a large fraction of their mass (but not a relaxed flat outer envelope, as was discussed previously^{18,19}). Tandem encounters of the same system tend to strip the outer parts and produce a smaller system, which retains a flat inner profile.

Most of the stripped stars in our simulations did not become bound to the perturber but rather escaped from the two-body system altogether. This indicates that stripped material from a realistic, N -body, perturber would not become bound to the perturbed system and, therefore, have no substantial effect on its mass profile. We therefore assume that

our results for a point perturber are, for similar p values, also qualitatively representative for an N -body perturber. This assumption is supported by the consistency of our results for the energy-gain and mass-loss with those obtained by other simulations of encounters between identical systems¹¹. The substantial amount of stripped material might remain bound to a larger group, forming a common background halo which might later infall and produce even more extended envelopes around the haloes.

If slow hyperbolic encounters are to be considered responsible for $M \propto R$ profiles, one should estimate how frequently they occur in a cosmological context. Tidal encounters are found to be effective whenever $p \leq 5 R_h$, so that the geometrical cross-section for such an encounter is much larger than that for a merger, which occurs for $p \leq 2.5 R_h$ (refs 9, 20, 21). The time scale for mergers in a small group is estimated analytically to be of the order of the dynamical time scale of the group²², and N -body simulations show that about one-third of the galaxies have undergone mergers, mostly in the first generation of their clustering. One may, therefore, conclude that the proposed mechanism is quite relevant in small groups.

A weak lower limit on the rate of encounters can be estimated for 'field' galaxies. A straightforward $n\sigma v$ calculation shows that the number, N , of effective encounters since the epoch of halo formation, Z_{form} , is

$$N \sim 0.05 \Omega_0 (1 + Z_{\text{form}})^2 \left(\frac{R_h}{60 \text{ kpc } h_{50}^{-1}} \right) \left(\frac{v}{300 \text{ km s}^{-1}} \right) \quad (2)$$

where v is the typical peculiar velocity of galaxies. Each quantity in equation (2) has uncertainties but N does seem to be a number of the order of unity. There might be an additional contribution to N from dark haloes which do not have visible cores (galaxies)¹³.

The suggested mechanism predicts a correlation between the degree of clustering of a galaxy (be it a field galaxy, a member of a small group or a member of a large one) and the fractional extension of its $M \propto R$ region. Note also that tidal encounters lead to haloes which are both less massive and have larger fractional $M \propto R$ regions, whereas cosmological infall should lead to larger $M \propto R$ regions in more massive haloes.

Tidal interactions might also be helpful in the merger picture for halo formation, as suggested by White and Rees²². There, each merger is preceded and followed by several tidal encounters which might reproduce inner $M \propto R$ regions even when the mergers themselves may steepen their profiles⁹.

Another important indication from our results is the tendency for complete removal of haloes after many encounters. This is seen from the fact that, as a result of a tidal encounter, each 'shell' of stars expands, so that most parts of the system become less bound, an easy target for further stripping. It is expected, therefore, that galaxies now in rich clusters have lost most of their haloes due to successive encounters during their clustering process, and that galaxies observed to have haloes could not have undergone too many tidal encounters.

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Interpretation of type I OH maser sources

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OH maser sources of type I, which are related to regions of star formation, have been known for more than a decade. However, despite great effort no satisfactory theoretical interpretation has yet been given. (For general reviews on cosmic masers see refs 1–5.) A model for the interpretation of type I OH maser sources is suggested here. This model combines the effect of velocity and magnetic field gradients with radiative pumping by means of the ($^2\Pi_{3/2}$, $J=5/2 \rightarrow ^2\Pi_{1/2}$, $J=5/2$) transition. Due to an overlap of particular hyperfine components, inversion of the 1,665 MHz line is favoured.

Type I OH maser sources are characterized by an extreme intensity corresponding to a radiation temperature up to 10^{13} K in one or both of the main lines, the 1,665 MHz-line usually being the stronger, and by a high degree of circular polarization (up to 90%).

Schemes involving optical pumping^{6–11}, chemical pumping^{12,13}, and collisional pumping (refs 12, 15, 16, and C. H. Townes and W. D. Gwinn, unpublished data) have been used to explain the intensities. Although all these mechanisms may produce population inversions, difficulties arise when a more quantitative interpretation of the observations is attempted. For example, consider a radiative pumping scheme: on one hand it is not easy (though possible) to have enough pump photons to explain the observed high luminosities in the radio lines, on the other hand it is difficult to have an efficient pump mechanism and a large absolute value of the optical thickness in the maser line at the same time, because the absolute value of the optical thickness of the pump line along the same line of sight is in general larger than that of the maser line⁴. Note also that the upper and the lower levels of either of the main lines are distinguished only by their parity, while all other quantum numbers are identical. Thus to every spectral line connecting to the lower level there is a corresponding line connecting to the upper level. Inversion can, therefore, come about only by difference effects and not by selection rules.

To explain the high degree of polarization a model has been proposed which invokes gradients of the velocity and the magnetic field^{17,18}. If the velocity and the magnetic field change along the line of sight, it can be hypothesized that in a certain region for a particular direction of propagation, the derivatives of the frequency shifts of the maser line due to the Doppler and the Zeeman effect just cancel each other out. This leads to a larger absolute value of the optical depth than one would have without Zeeman splitting.

If κ is the absorption coefficient

$$\kappa = \kappa_0 \exp \{ -(\Delta v / \Delta v_{\text{th}})^2 \} \quad (1)$$

we estimate the optical thickness in the line by

$$\tau = \kappa_0 \Delta l \quad (2)$$

where Δl is the path length along the line of sight over which the frequency shift changes by an amount corresponding to the thermal width Δv_{th} of the line. Using a Taylor expansion for the radial velocity (v_r) and the magnetic field (H), we obtain for the frequency shift along the line of sight:

$$\Delta v \approx (\Delta v)_0 + \left[\frac{v_0}{c} \frac{dv_r}{dl} + \gamma \frac{dH}{dl} \right] \cdot \Delta l + \frac{1}{2} \left[\frac{v_0}{c} \cdot \frac{d^2 v_r}{dl^2} + \gamma \frac{d^2 H}{dl^2} \right] \cdot (\Delta l)^2 \quad (3)$$

($\gamma = 3.31 \text{ MHz Oe}^{-1}$ for the 1,665 MHz-line and 1.99 MHz Oe^{-1} for the 1,667 MHz-line.)

For those lines for which the Zeeman effect is not important we can estimate Δl from the linear term in equation (3):

$$\Delta l_1 = \left| \frac{v_{th}}{dv_r/dl} \right| \quad (4)$$

If, however,

$$\frac{v_0}{c} \frac{dv_r}{dl} + \gamma \frac{dH}{dl} = 0 \quad (5)$$

the linear term in equation (3) cancels and we have to determine Δl from the second-order term:

$$\Delta l_2 = \left| \frac{2v_{th}}{\frac{d^2 v_r}{dl^2} + \frac{c\gamma}{v_0} \frac{d^2 H}{dl^2}} \right|^{1/2} \quad (6)$$

As γ has a different sign for the different Zeeman components, this increase in the optical depth occurs only in one of the Zeeman components while in the other there is a decrease.

Thus, if this mechanism were operating, one would expect the maser radiation to consist mainly of only one of the Zeeman components. Observations show that in most cases the right hand and the left hand circularly polarized radiation come from different volume elements.

We suggest that type I OH masers may be due to a combination of the above effect with a particular scheme of optical pumping. For this we note that the relative Zeeman splitting ($\Delta\lambda/\lambda$) is much larger for the radio lines than for the IR lines. Thus, if condition (3) is satisfied for the maser line, the same cannot be true for the IR lines. Therefore we can apparently construct a model in which the absolute value of the optical depth in the maser line is large while that in a potential pump line stays at moderate values ($\tau \sim 1$).

To achieve the pumping we consider the effect of overlap of IR-lines; such pumping schemes have been suggested elsewhere^{19,20}. Note that any IR-pumping scheme which is to invert the main lines has to use transitions with $\Delta J=0$. Among these, the transitions connecting the states with $J=5/2$ have the largest overlap and seem therefore to be the most important. According to the frequencies given by Destombes *et al.*²¹, in the ($^2\Pi_{3/2}$, $J=5/2$) $\rightarrow$ ($^2\Pi_{1/2}$, $J=5/2$) multiplet there is an almost complete overlap between two main lines, the ($F=3^- \rightarrow F=3^+$) and the ($F=2^- \rightarrow F=2^+$) transitions. The frequency difference is 0.5 MHz, corresponding to $\Delta v/v \approx 8 \times 10^{-8}$, which is considerably less than the line width for any reasonable temperature. In contrast, the frequency difference between the ($F=3^+ \rightarrow F=3^-$) and the ($F=2^+ \rightarrow F=2^-$) transitions is 57.5 MHz corresponding to $\Delta v/v = 0.9 \times 10^{-5}$, which is much wider than typical line widths in OH-maser sources (see Fig. 1). Thus the optical depth in the first pair of lines is about twice that for the latter. If we now consider the radiation from an IR-source outside the OH-cloud, at a point

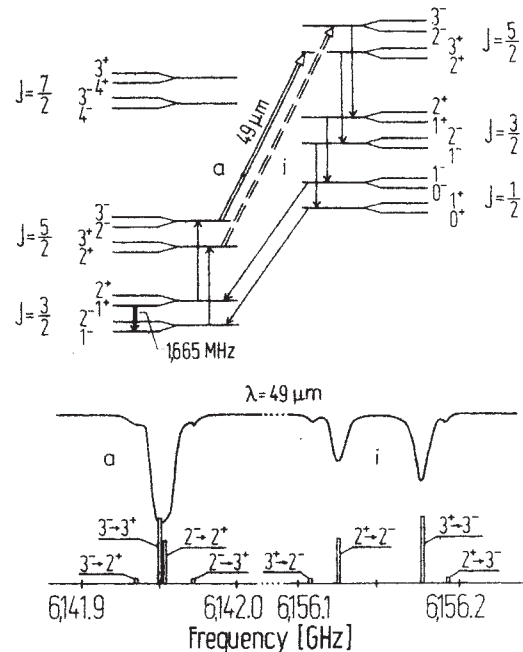


Fig. 1 Energy level diagram of the OH molecule (not to scale) and spectrum of the 49 μm multiplet.

within the cloud at which the optical thickness in the non-overlapping IR-lines is about unity, the intensity in the overlapping lines is about one-third of that in the non-overlapping ones. Thus the rate of absorption from the lower half of the ($^2\Pi_{3/2}$, $J=5/2$) Λ -doublet is ~ 6 times larger than that from the upper half. (A factor of two comes from the fact that the non-overlapping lines cover a wider frequency range.) This effect produces a population inversion in the ($^2\Pi_{3/2}$, $J=5/2$)-state as well as in the ($^2\Pi_{1/2}$, $J=5/2$)-state. Both are transferred to the ground state by the other IR-transitions.

The total number of absorbed pump photons is proportional to $L_{v,IR} \cdot \Delta v_p$. The maser region is determined by Δl_2 (equation (6)). As the Zeeman effect is not important for the IR-lines, Δv_p is much wider than the thermal line width and we can approximate

$$\Delta v_p = \frac{v_{p,0}}{c} \frac{dv_r}{dl} \Delta l_2 = v_{p,0} \frac{v_{th}}{c} \frac{\Delta l_2}{\Delta l_1} \quad (7)$$

$L_{v,IR}$ may be estimated either from models for IR-sources or from measurements. For W3(OH) $L_{v,IR}$ was determined²² to be of the order of $4 \times 10^{25} \text{ erg s}^{-1} \text{ Hz}^{-1}$ at $\lambda = 49 \mu\text{m}$.

We now make some quantitative estimates using an oversimplified model. As type I OH-masers occur in regions of star formation, it seems reasonable to consider a collapsing cloud with a central IR-source. If we assume that the kinetic energy density is larger than the magnetic energy density everywhere, the hydrodynamics of the collapse is essentially not altered by the magnetic field and we may assume radial symmetry (the magnetic field lines will also be essentially radial).

For free infall, we have

$$v(r) = v_0 (r_0/r)^{1/2} \quad (8)$$

$$n(r) = n_0 (r_0/r)^{3/2} \quad (9)$$

$$H(r) = H_0 (r_0/r)^2 \quad (10)$$

In this model condition (3) is satisfied if

$$r^{1/2} (3p^2 - r^2) = \alpha (r^2 - p^2)^{1/2} \quad (11)$$

where p is the impact parameter of the line of sight and

$$\alpha = \frac{4c\gamma H_0 r_0^{3/2}}{v_0 v_0} \quad (12)$$

For simplicity we consider the central ray ($p=0$) only. Along this the maser region is around r_m which is determined from equation (11) to be

$$r_m = (-\alpha)^{2/3} \quad (13)$$

With $p=0$ we find

$$\frac{\Delta l_2}{\Delta l_1} = \left[\frac{2v_0 r_0^{1/2}}{3v_{th} (-\alpha)^{1/3}} \right]^{1/2} \quad (14)$$

This ratio, which is independent of r_0 , gives the increase of the optical depth of the maser line over the value without Zeeman effect. It also corresponds to the ratio Δv_p to the thermal width of the pump line. If we assume $v_0 = 5 \text{ km s}^{-1}$, $H_0 = 10^{-3} \text{ Oe}$ and $T_{\text{kin}} = 50 \text{ K}$, which seem to be reasonable parameters, we have $\alpha = 0.48 r_0^{3/2}$ and $\Delta l_1/\Delta l_2 \approx 4.4$.

We estimate the number of available pump photons on one side by

$$W_{\text{ph}} \approx 4\pi r_s^2 \cdot \pi B_\nu(T_s) \Delta\nu_p \quad (15)$$

where r_s and T_s are the radius and the temperature of the central IR-source. With $r_s \approx 10^{15} \text{ cm}$ and $T_s \approx 300 \text{ K}$ we obtain $W_{\text{ph}} \approx 10^{44} \text{ photons s}^{-1}$ which seems to be about of the right order of magnitude. If, on the other side we estimate W_{ph} from the IR-luminosity measured²² for W3(OH), we obtain about two orders of magnitude more photons. For these photons to be absorbed, the optical depth in the pump lines has to be about unity, that is we have to have $\Delta l_2 \kappa \approx 1$. With the above assumed parameters this leads to the requirement that $n_{\text{OH}} \cdot r_0 \approx 10^{17} \text{ cm}^{-2}$. With $r_0 \approx 10^{16} \text{ cm}$ this requires an OH density of about 10 cm^{-3} or with $n_{\text{OH}}/n_{\text{H}_2} \approx 10^{-5}$ a hydrogen density of $\sim 10^6 \text{ cm}^{-3}$, which seem to be reasonable.

These estimates show that the proposed scheme may indeed explain the observed properties of type I OH-sources. In particular it explains: (1) the large differences in the relative intensities for the different subsources, these are because the intensity depends on the gradients of the radial velocity and the magnetic field along the line of sight, which vary much more than other physical parameters; (2) the high degree of polarization; (3) why in general the 1,665 MHz-line is more intense than the 1,667 MHz-line. This is expected if in the maser region the number of $49 \mu\text{m}$ -photons absorbed in the ($F=2^+ \rightarrow F=2^-$) transition is larger than that absorbed in the ($F=3^+ \rightarrow F=3^-$) transition. This is based on the lowest order consideration in which the satellite transitions ($\Delta F \neq \Delta J$) in the IR-lines are neglected. In this approximation the pump routes for the 1,665 MHz and the 1,667 MHz-line are decoupled and may be considered independently of each other. The number of photons absorbed in a line per cm^3 per s is $\kappa I \Delta\nu$ with $I = I_0 e^{-\tau}$. If we further assume that the ratio of the occupation numbers of the different hyperfine components of the ($2^1\Pi_{3/2}$, $J=5/2$)-state corresponds to that of their statistical weights (7/5), the ratio of the absorption coefficients of the pump lines is also given by that number. It then follows, that the number of photons absorbed in the ($F=2^+ \rightarrow F=2^-$) transition of the $49 \mu\text{m}$ -line is larger than that of the ($F=3^+ \rightarrow F=3^-$) transition if the optical thickness τ of the latter is larger than 0.31. In more detailed investigations, however, one has to consider the complete multi-level system containing all levels and transitions possibly involved in the pumping scheme.

From the conservation of angular momentum, the IR-lines which provide the pumping would also be expected to be circularly polarized.

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Determination of ^{129}I using tandem accelerator mass spectrometry

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Iodine-129, with a half life of 15.7 Myr (ref. 1), is one of the longest lived of the cosmogenic radionuclides. Although the primordial supply of ^{129}I is now extinct, ^{129}I is continuously being produced in the atmosphere primarily by cosmic ray reactions on xenon², in the Earth primarily by spontaneous fission of ^{238}U (ref. 3), and in meteorites and the Moon primarily by proton and neutron induced reactions on Te and Ba (ref. 4). The relatively large quantity of ^{129}I introduced into the environment from the nuclear age may be useful as a tracer in groundwater hydrology⁵. Fissionogenic ^{129}I in large granite formations (batholiths) could be used to establish the suitability of these sites for long term nuclear waste storage, because groundwater movement would carry away the more soluble iodides and disturb the equilibrium $^{129}\text{I}/^{238}\text{U}$ ratio. The concentration of ^{129}I in meteorites can be combined with results for shorter-lived radionuclides to provide information on the constancy of the galactic cosmic ray flux over longer time scales than previously possible and also on the preterrestrial history of meteorites. We have applied the new atom counting technique to ^{129}I analysis with a sensitivity (the minimum number of atoms in the sample required to obtain a quantitative result) of less than 10^7 atoms of ^{129}I in a 1-mg sample. An AgI standard with a known $^{129}\text{I}/^{127}\text{I}$ ratio of 10^{-11} was determined to $\pm 10\%$, and the background contribution from a reagent grade AgI sample gave an upper limit to the ratio of about 3×10^{-13} . We used a time-of-flight measurement to distinguish ^{129}I from the stable ^{127}I ions that were not separated by the mass analysis system. Our results for the meteorites Bruderheim and Dhajala represent the first direct (that is, not inferred⁶ from radiogenic Xe) determination of ^{129}I in meteorites. A Xe molecular negative ion was discovered but we show that it is not a problem for ^{129}I analysis.

Because of its long half life, ^{129}I measurement by counting its β decay is not very sensitive and is useful only for samples with high activity⁷. Although our mass spectrometry measurements of ^{129}I are the first to use an accelerator, McHugh and Sheffield⁸ used a three stage mass spectrometer with a negative ion sputter source to detect carrier free ^{129}I with a sensitivity near 10^9 atoms for samples with a $^{129}\text{I}/^{127}\text{I}$ ratio of about 10^{-7} . Their measurements were limited by interference from the ^{127}I tail and from molecular ions near mass 129 such as $^{97}\text{MoO}_2^-$ which could not be differentiated from $^{129}\text{I}^-$.

The neutron activation technique has been quite successful, with numerous reports^{3,9} of ^{129}I measurements in natural samples with sensitivities typically in the range 10^{10} – 10^{12} atoms. The neutron activation technique involves counting 12-h ^{130}I produced from the $^{129}\text{I}(\text{n},\gamma)^{130}\text{I}$ reaction in a high flux reactor. A careful chemical pre-separation is used to reduce interference from reactions such as $^{133}\text{Cs}(\text{n},\alpha)^{130}\text{I}$ and induced fission on uranium. Interference from 13-day ^{126}I produced by $^{127}\text{I}(\text{n},2\text{n})$ often limits the sensitivity^{2,3}, and for this reason Rook *et al.*¹⁰ followed neutron activation with isotope separation of mass 130 and then used β - γ coincidence counting of ^{130}I . The sensitivity was improved somewhat for samples with high ^{127}I content but this procedure still does not discriminate against ^{130}I produced from trace impurities and from the $^{127}\text{I}(3\text{n},\gamma)^{130}\text{I}$ multistep reaction². By contrast, our method offers simplified sample preparation, a single analysis step taking <1h, no interference from impurities, background only from the ^{127}I tail, and a potential improvement in sensitivity by several orders of magnitude. This will allow ^{129}I measurements in considerably smaller samples, and will not require the use of natural iodine concentrators such as animal thyroids, seaweed, and brine.

The apparatus used in the present work is the same as that used for ^{36}Cl (ref. 11) and ^{26}Al (ref. 12) except that the final ionization detector was replaced with a 2.2-m time-of-flight (TOF) detector. The ^{129}I can be identified on the basis of mass alone as the only stable isobar of ^{129}I is ^{129}Xe , a noble gas that is not expected¹³ to form negative ions (but see below). However, interference is expected from the stable ^{127}I , two mass units away. The I^- beam (1–5 μA) produced from NaI or AgI samples in the Cs sputter source was accelerated to the 5 MV tandem terminal, stripped of several electrons, then accelerated again to ground potential. Charge state +5 ions with an energy of 30 MeV were selected by the 90° analysing magnet and 10° electrostatic deflector. These analysers eliminated all molecules and gave a combined resolution in M/q of $\sim 0.5\%$ FWHM. Although the ^{129}I is thus separated by 3 FWHM from the ^{127}I , a combination of charge exchange and scattering in the acceleration tubes and analysers allowed up to 200 counts per s (c.p.s.) of ^{127}I to pass into the detector. As the interfering $^{127}\text{I}^{5+}$ ions passed two

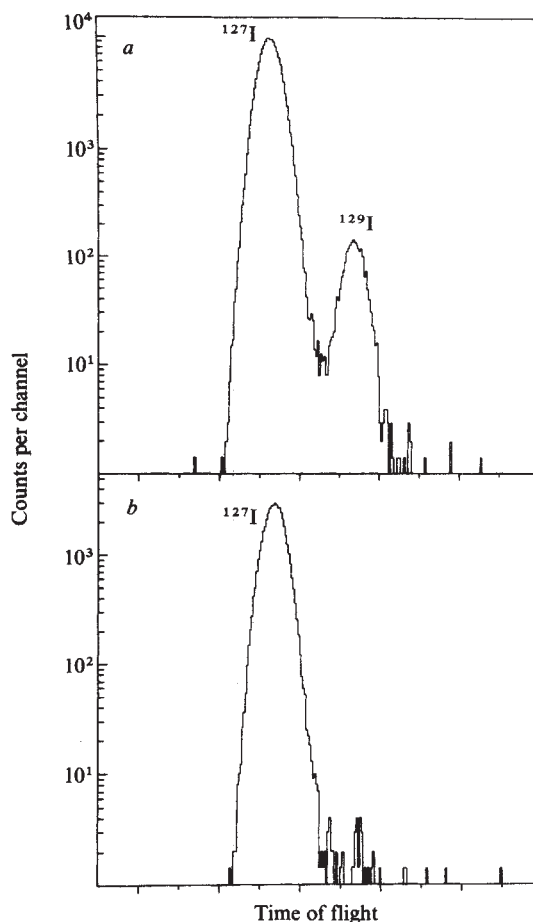


Fig. 1 Time-of-flight spectra for runs on: a, an AgI sample enriched to $^{129}\text{I}/^{127}\text{I} = 10^{-11}$; b, AgI made from reagent grade NaI. These events have been gated with a window on the total energy signal. The peaks are separated by 5.2 ns and the overall time resolution is 1.2 ns FWHM.

magnetic analysers, most of them had the same magnetic rigidity (ME/q^2) as the $^{129}\text{I}^{5+}$ ions at the detector and therefore differed by 1.5% in energy (E) and in velocity ($\propto (E/M)^{1/2}$). Heavy-ion detectors are not available with an energy resolution adequate to resolve ions at this mass and energy, so we measured the velocity with TOF. The start signal was obtained^{14,15} from the electrons produced by the iodine ions passing through a $5 \mu\text{g cm}^{-2}$ carbon foil; the stop signal was obtained from a 100-mm² area Si surface barrier detector. The Si detector also gave an energy signal adequate to resolve ions in lower charge states having nearly the same E/q and velocity.

TOF spectra for runs on the standard and blank (Fig. 1) show that the ^{127}I peak is adequately resolved for samples having an $^{129}\text{I}/^{127}\text{I}$ ratio down to about 10^{-12} . We determined that the apparent levelling off of the ^{127}I tail at the ^{129}I location in the TOF spectrum for the blank was not due to ^{129}I or ^{129}Xe in the sample by moving the analysers off the mass 129 peak and noting that the intensity of the tail remained a factor of $\sim 10^3$ below the ^{127}I peak. The ^{127}I rejection factor for each of the analysis stages was approximately as follows: inflection magnet, 3; 90° magnet, 10^5 ; electrostatic analyser, 10^4 ; TOF, 10^3 .

Although Xe^- is probably unstable, XeF^- has been accelerated in tandems (H. E. Wegner and P. Thieberger, personal communication) and xenon may form other stable or metastable molecular negative ions. As Xe is a likely contaminant in the samples and in the residual gas of the ion source, we felt that it was important to determine whether there is any ^{129}Xe contribution to the mass 129 peak. With the analysis system set for ^{129}I from the reagent AgI blank, pure Xe gas was introduced into the source until the pressure increased a factor

Table 1 Measured ^{129}I concentrations

Sample	AgI weight (mg)	Counting time (min)	Total ^{129}I (counts)	$^{129}\text{I}/^{127}\text{I}$ (10^{-12} g per g)	129 atoms in sample
NSRL-20003, enriched AgI	~ 10	89	3052	$9.0 \pm 0.6^\dagger$	4.3×10^8
NSRL-20304, reagent blank AgI	~ 10	10	39	< 0.3	$< 1.4 \times 10^7$
NSRL-21601, chemistry standard*	1	27	203	$8 \pm 2^\dagger$	4.7×10^7
NSRL-21701, Bruderheim*	1	48	160	$6 \pm 2^\dagger$	2.8×10^7
NSRL-21801, Dhajala*	1	33	88	$7 \pm 2^\dagger$	3.3×10^7

*The two meteorite samples were obtained from 6.74 g and 22.2 g of Bruderheim and Dhajala. For these and the chemistry blank ~ 1 mg of iodine carrier and 5 mg of AgCl were added.

† The two enriched samples (ICN Cat. no. 67325) had an expected $^{129}\text{I}/^{127}\text{I}$ ratio of $(9.8 \pm 0.5) \times 10^{-12}$.

‡ The meteorite results for the $^{129}\text{I}/^{127}\text{I}$ ratio were normalized to the chemistry standard.

of two to 5×10^{-5} torr. The mass 129 counting rate then increased from 1 c.p.m. (which corresponded to a background level of $^{129}\text{I}/^{127}\text{I} = 6 \times 10^{-13}$) to 5 c.p.m. Further optimization with a source geometry more appropriate for a gas sample led to a maximum counting rate of 3,500 c.p.m., and this dropped by more than a factor of 100 soon after the gas was turned off. A mass scan with the inflection magnet (which directly follows the ion source) revealed that the Xe was being injected into the tandem as a molecular negative ion with $M/q = -131$ (such as $^{129}\text{XeH}_2^-$) although the $^{129}\text{Xe}^-$ ion could not be completely ruled out because of the limited resolution of the inflection magnet. (The resolution of the post-acceleration analysis and TOF were adequate to positively identify mass 129, charge state 5 ions. This, together with the high counting rate that was correlated with Xe pressure, is strong evidence that we were counting only $^{129}\text{Xe}^{5+}$). The counting rate was low compared to what might be expected from the $\sim 10^{10}$ Xe atoms being exposed to Cs in the sputter source. This can be explained if the probability of forming the (molecular) negative ion is low, if the cross-section for destruction in electric and magnetic fields is high, or if the ion is metastable with a life time short compared with the $15 \mu\text{s}$ trip to the terminal of the tandem. We estimate that if the residual gas in the source contains Xe in atmospheric concentrations (1.1 p.p.m.) and the Xe content of the samples is low (< 1 p.p.m.) then the ^{129}Xe will not contribute to the ^{129}I background until a sensitivity for ^{129}I of one part in 10^{18} is reached.

The $^{129}\text{I}/^{127}\text{I}$ ratios (Table 1) were obtained by cycling the magnets between the two isotopes: the ^{129}I was counted for 3–5 min, then the $^{127}\text{I}^{5+}$ current was measured in a Faraday cup following the 90° analyser. The transmission efficiency of the TOF detector ($\sim 30\%$) was continuously monitored with the ^{127}I ions. The 1σ errors are estimated from the scatter in the individual cycles and reflect both statistics and random fluctuations in the ion source output and accelerator transmission. Systematic errors such as those due to mass fractionation and current calibration contributes an additional uncertainty of $\sim 20\%$ to results for the standards, but most of these effects cancel out for the meteorite results because they are reported as ratios to the standard.

Preparation of the meteorite samples and an interpretation of their ^{129}I content will be subjects of another paper. The results given in Table 1 demonstrate that 10–20 g of meteorite are adequate for detailed ^{129}I analysis. The upper limit for the $^{129}\text{I}/^{127}\text{I}$ ratio of 0.3×10^{-12} for the reagent blank implies that the background level for a 1 mg AgI sample corresponds to less than 2×10^6 atoms of ^{129}I .

We are presently modifying the apparatus to reduce the ^{127}I tail and increase the sensitivity. A new inflection magnet with a resolution $M/\Delta M$ of better than 150 will reduce the number of $^{127}\text{I}^-$ ions that are injected into the accelerator by 2 or 3 orders of magnitude and will also reduce the contribution from a Xe molecular negative ion. This magnet, together with improved vacuum in the electrostatic analyser, should reduce the ^{127}I interference so that it no longer limits the sensitivity. At the same time the sensitivity will be further improved by increasing the $^{127}\text{I}^-$ beam current to over $10 \mu\text{A}$ with a new ion source and by obtaining better transmission in the TOF detector. We anticipate being able to measure $^{129}\text{I}/^{127}\text{I}$ ratios in 1-mg iodide samples below 10^{-15} , a level that has already been reached with ^{36}Cl detection¹⁶.

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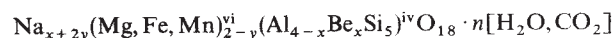
Channel CO_2 in cordierites

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The formulae commonly cited for cordierite usually ignore the occupants of the structural channels that run parallel to the c -axis. Recently, however, it has been shown¹ that two of the more common channel occupants, Na^+ and molecular H_2O , significantly modify cordierite's refractive indices (α, β, γ), optic angle ($2V_x$), unit cell dimensions, and the distortion index (Δ) defined by Miyashiro². Indeed, for 11 natural cordierites, excluding indialites, Δ correlates almost entirely with channel Na^+ and/or H_2O and with R_s , the average radius of the ions occupying the octahedral sites, but not (as long believed) with Al/Si ordering. After heating at 800°C for 6 h, Δ increased for the four crystals remeasured, and $2V_x$ increased for the nine optically (–) crystals but decreased to below 90° for the two optically (+) crystals. Selkregg and Bloss³ attributed these effects to loss of channel H_2O and/or Na^+ but did not explain why the two (+) crystals became (–) after heating. We report here that loss of molecular CO_2 , a frequently reported channel occupant^{3–5}, was the reason. We observe that for Mg-cordierite, $2V_x$ is $\sim 87^\circ$ if its channels are vacant, decreases to 45° or less as channel H_2O increases and increases to 114° with increasing channel CO_2 (but no H_2O). Channel CO_2 is apparently the long-sought-for cause of optically (+) cordierites.

The previously unexplained occurrences of (+) and (–) cordierites on opposite sides of a fault⁶ probably represent control of CO_2 distribution by the fault. The seemingly capricious variation of $2V_x$ for cordierites with comparable Mg/Fe ratios, probably results from channel occupancy by varying proportions of H_2O and CO_2 and, perhaps, other channel occupants. In view of the importance of the channel occupants, the formula for cordierite should perhaps be



where y allows for vacancies in the octahedral sites^{1,7} which, like substitution of Be^{2+} for Al^{3+} , seem to correlate with channel Na^+ . The formula does not accommodate the tetrahedral vacancies reported by Wallace and Wenk⁷, or additional channel occupants, but these might be relatively minor in amount and effect.

Cordierite occurs in a wide variety of metamorphic and igneous rocks and, by its presence or absence, conveys information regarding their conditions of formation. In the ceramic industry, its easy synthesis and high resistance to thermal shock favour its use in spark plugs, catalytic supports to treat automobile exhaust and, in the future, for fabrication of turbine engines. Consequently, solution of the long-standing

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mystery of why some cordierites are optically (+) but most are (−) seemed important because the factors involved, whether chemical or structural, might influence the stability and conditions of formation of cordierite.

Spindle stage studies⁸ of 20 natural cordierites disclosed five to be optically (+) with $2V_x > 90^\circ$. Microprobe analyses revealed no significant compositional differences that correlated with optic sign. However, IR spectra between 200 cm^{-1} and $4,000\text{ cm}^{-1}$ exhibited not only H_2O bands, but also CO_2 absorption bands near $2,350\text{ cm}^{-1}$. These were intense for the (+) cordierites but weaker for the (−) cordierites. Refractive indices measured to 0.0005 by the double variation method disclosed that the (+) cordierites possessed higher refractive indices and birefringence ($\gamma-\alpha$) compared with optically (−) crystals with the same iron content. When heated to $800\text{--}1,200^\circ\text{C}$ to expel the more volatile channel occupants, $2V_x$ increased for the (−) crystals but decreased for the (+) crystals.

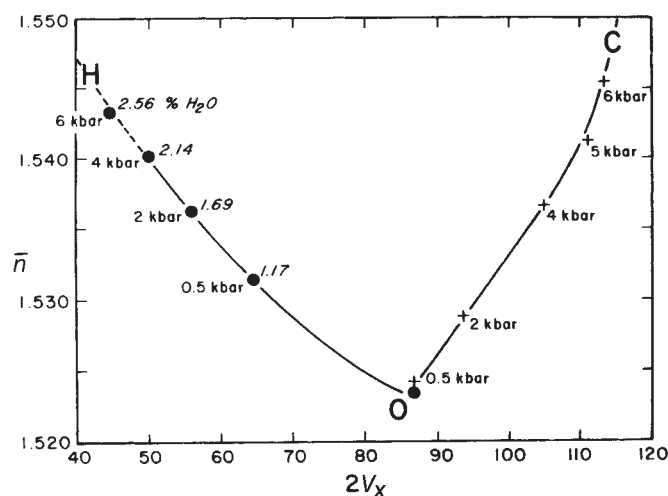


Fig. 1 Plot of the average index $\bar{n}$ [$\bar{n} = (\alpha + \beta + \gamma)/3$] versus $2V_x$ (averaged for wavelengths 400, 666 and 900 nm) for White Well cordierite after tempering at $1,300^\circ\text{C}$ for 30 h (point O), for this same material after CO_2 was forced into it at increasing CO_2 pressures (plus signs on line OC), and after H_2O was forced into it (● on line HO). The weight percentages of H_2O were estimated using the equations of Medenbach *et al.*⁹. Weight percentages of CO_2 are yet to be determined but the increase in $\bar{n}$ clearly shows that increasing quantities of CO_2 are being forced into the channels as CO_2 pressure increases.

The influence of channel CO_2 and H_2O on the optical properties of Mg-cordierite was more directly investigated using a (−) cordierite from White Well, Australia ($a = 17.065\text{ \AA}$, $b = 9.724\text{ \AA}$, $c = 9.346\text{ \AA}$; optic orientation, $a = Z$, $b = Y$, $c = X$). This specimen's optical properties were measured before and after tempering for 30 h at $1,300^\circ\text{C}$ in air to expel channel CO_2 and H_2O . Crystals thus tempered were also examined after being annealed for 30 days at 600°C and at various pressures of H_2O and of CO_2 (from decomposition of $\text{Ag}_2\text{C}_2\text{O}_4$). The results (Fig. 1) clearly reveal the opposite effects on $2V_x$ of CO_2 and H_2O ; however, both increase the average index $\bar{n}$ [$\bar{n} = (\alpha + \beta + \gamma)/3$]. Surprisingly, IR spectra of the tempered material taken before its rehydration or recarbonation revealed Si–OH groups and/or H_2O that either survived the $1,300^\circ\text{C}$ heating or re-entered after heating. Microprobe analyses showed that about half of the channel Na had also survived this heating.

Table 1 reveals that the birefringence ($\gamma-\alpha$) increases significantly with CO_2 and that the distortion index Δ decreases significantly with channel H_2O and CO_2 . Thus

decarbonation of a CO_2 -rich cordierite by heating will cause Δ to increase whereas $2V_x$ and ($\gamma-\alpha$) will decrease. This inverse correlation between $2V_x$ and Δ as channel CO_2 increases thus opposes the statement of Miyashiro² that, in general, the larger Δ , the larger $2V_x$ will be.

Table 1 Optical properties, cell edges* and distortion index Δ for the White Well cordierite†

	Natural	Tempered	Carbonated at 4 kbar	Hydrated at 4 kbar
α	1.5345	1.5235	1.5326	1.5386
c (Å)	9.346	9.340	9.348	9.346
β	1.5392	1.5254	1.5381	1.5440
b (Å)	9.724	9.722	9.723	9.725
γ	1.5423	1.5275	1.5456	1.5450
a (Å)	17.065	17.076	17.059	17.060
$(\gamma-\alpha)$	0.0078	0.0040	0.0130	0.0064
Δ	0.243°	0.260°	0.241°	0.237°
$2V_x \dagger$	76.4°	87.0°	105°	50.0°

*From back reflection Weissenberg photographs.

†Optic orientation, $c = X$, $b = Y$, $a = Z$.

‡Average of measurements at 400, 666, and 900 nm.

IR spectra for single cordierite crystals³ were later interpreted⁴ as showing that 80% of the channel CO_2 molecules were aligned perpendicular to c ($=X$) with the other 20% possibly aligned parallel to c . The increases in refractive indices produced by carbonation of the tempered White Well cordierite— $\Delta\alpha = 0.0091$, $\Delta\beta = 0.0127$, $\Delta\gamma = 0.0181$; average increase 0.0133—suggest a dominant alignment of CO_2 parallel to the a -axis ($=Z$) because $\Delta\gamma$ significantly exceeds the average index increase of 0.0133. Alignment of CO_2 molecules parallel to the c -axis is not supported by this present evidence because $\Delta\alpha$ ($=0.0091$) is significantly below the average increase of 0.0133.

The marked increase in refractive indices with channel CO_2 for cordierite suggests that H_2O contents, if calculated using the measured refractive indices and Fe contents for cordierite¹⁰, will be too high when significant CO_2 is also present. Work in progress indicates that the amounts and relative proportions of channel H_2O and CO_2 will be readily determinable for single crystals of cordierite if spindle-stage measurements of $2V_x$ and refractive indices are followed by microprobe analyses for Na, Fe, Mg, and Mn. We know of no other technique, so relatively simple and routine, that can determine the same data for a single grain. Knowledge of the relative proportions and amounts of the volatile species trapped in the channels of cordierite has potential use as an indicator of metamorphic and magmatic fluids.

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A fault plane solution for the Carlisle earthquake, 26 December 1979

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Deformation at plate boundaries is well determined from the fault parameters of large earthquakes but within plates relatively little is known. Although other methods of measuring *in situ* stress are available only intra-plate earthquakes provide information about stresses at depths of several kilometres¹. Accurate location and the determination of fault parameters for intra-plate earthquakes is difficult because such earthquakes are rare and generally too small to be widely recorded. This problem has been overcome in parts of the eastern USA by installing permanent dense seismometer networks, and brief aftershock studies of the type described here. Our aftershock study has provided an accurate location of a significant UK earthquake, and a reliable measure of compression direction in a region of Europe distant from the influence of Alpine motion.

On the 26 December 1979 at 03h 57min an earthquake occurred north of Carlisle. The same day the Institute of Geological Sciences (IGS) in Edinburgh reported the shock to be $m_b=5.0$ and probably located close to Longtown. Their assessment of epicentral position was based on the occurrence, earlier in 1979, of three smaller shocks (now taken to be foreshocks) which occurred in the area. The region has some history of activity with an earthquake occurring in 1786 which was felt at distances of 300 km (similar to the recent earthquake) and a sequence of four earthquakes in 1901 (ref. 2).

Damage from the earthquake was slight but created wide publicity and interest. A preliminary survey of intensity was determined by telephoning local police stations in the area and

confirmed the IGS view that an epicentre near Longtown was probable. On the basis of the foregoing evidence, an aftershock study using Sprengnether MEQ800 smoked drum recorders was initiated. Eight instruments were taken by car to Carlisle, arriving in heavy rain and floods, and one instrument was installed at 1800 GMT on the same day as the earthquake. A small earthquake at 05.39 on 27 December confirmed, with a 1.1 s S-P time, that the aftershock sequence was likely to be close to Longtown. An array of instruments indicated in Fig. 1 was deployed. A maximum of seven stations were operated simultaneously. Station 1 was moved because of site noise. Other changes in instrument disposition were carried out in response to preliminary locations with a view to optimizing location accuracy and fault plane solution resolution.

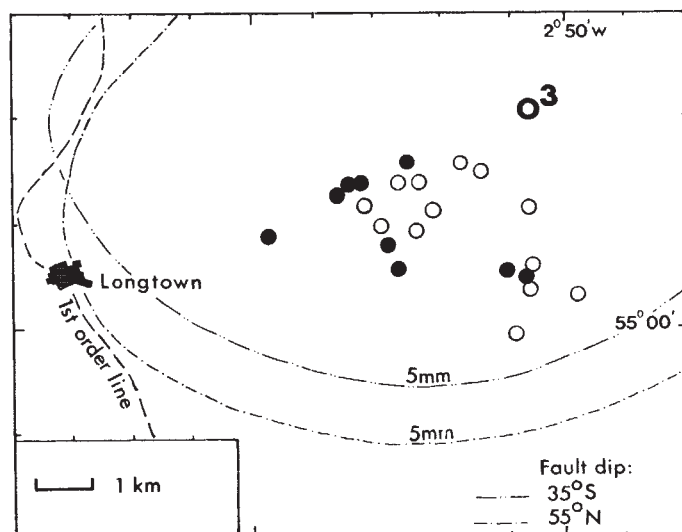


Fig. 2 Epicentral locations. ●, Shocks located with an error less than 1 km; ○, shocks located with an error less than 2 km. The 5-mm contour of predicted uplift is shown for both possible fault planes. The route of a first-order levelling line is also shown. The velocity model used for the locations and the fault plane solution was marginally the best of a set used to test the stability of the locations. It had a 3.3 km s^{-1} layer 1-km thick, a 5.0 km s^{-1} layer 4-km thick, a 5.7 km s^{-1} layer 15-km thick and the Moho velocity was taken to be 8.0 km s^{-1} . Because of the obvious lateral variation of the geology of the region on a scale comparable with the size of the seismograph array and the model insensitivity of the location procedure, the model adopted has no definite geological significance.

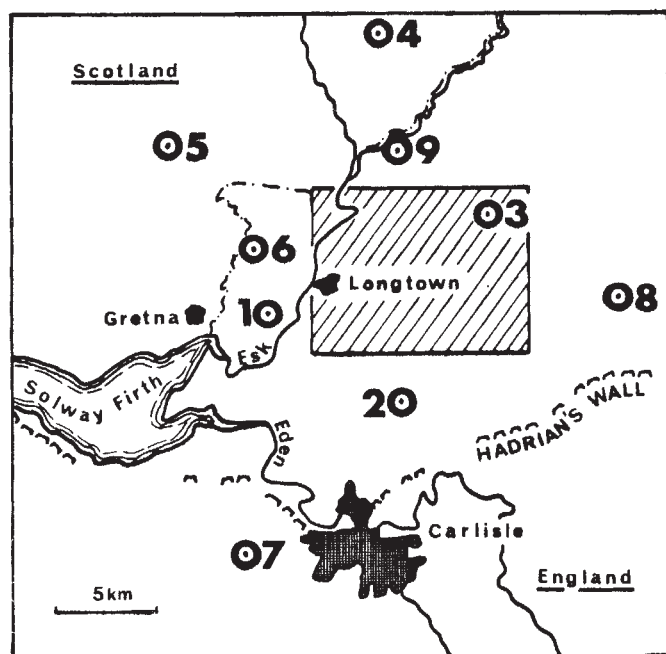


Fig. 1 The area of the survey showing station locations. The shaded area is enlarged in Fig. 2.

Selecting good sites proved impossible with many seismometers placed in the muddy middles of rain flooded fields. This reduced noise from rural roads and noise from the many trees and bushes. However, despite these difficulties, good low noise records were obtained. During the evening of 27 December temperatures dropped well below freezing and the ground remained frozen until after the stations were removed. The improvement of coupling was substantial and some seismometers were chipped out of the ice with difficulty. There was no wind during the recording period and cultural noise was generally low, only increasing on the 2 January 1980; 1 January, 1980 was particularly quiet. Low noise at this time was particularly fortunate as a major burst of seismic activity started in the early hours of the New Year with the major aftershock ($m_b=3$) of the sequence occurring at 05.06 GMT.

Twenty-seven earthquakes were recorded on four or more stations. Of these 22 provided satisfactory hypocentral locations (with errors less than 2 km) and nine were located to

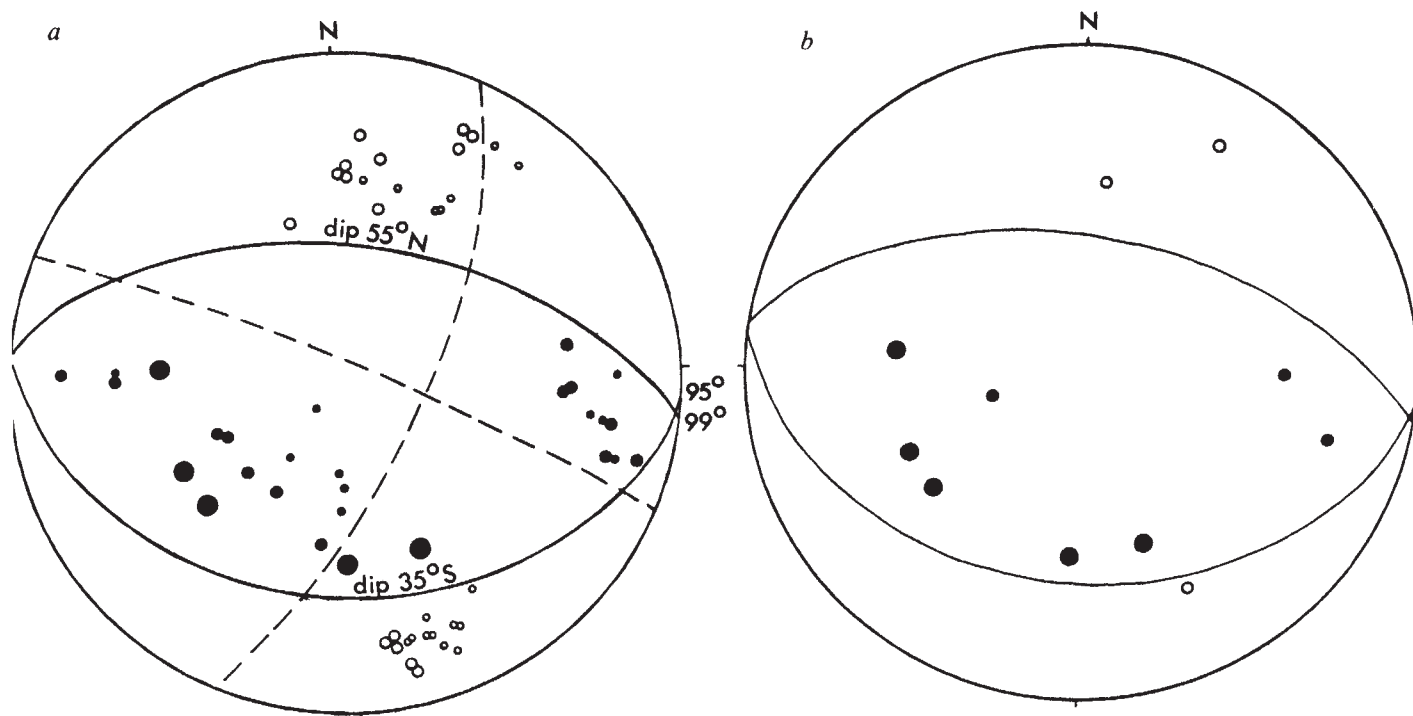


Fig. 3 *a*, Composite fault plane solution for the earthquakes shown in Fig. 2. Large circles, well located earthquakes; small circles, moderately located earthquakes: solid circles, compressions; open circles, dilatations. The very large circles are distant stations, and are all compressional. These latter data constrain the solution to be thrusting shown by solid lines and not strike-slip shown by dotted lines (the aftershock distribution also supports this conclusion). The nodal planes strike at 99° or 95° with dips of 35°S or 55°N respectively. *b*, The fault plane solution using only data for the aftershock at 05.06 GMT on 1 January 1980.

within 1 km. Location was carried out using the HYP071 location program³ using a rough estimate of velocity structure. No reliable information is available to constrain the shallow velocity structure apart from a general knowledge of the geology and travel time residuals in the location process. However, even velocity structures that caused systematic station residuals to appear did not change most locations by more than 1 km.

Many first motions were clearly readable. These are shown as a composite fault plane solution in Fig. 3*a*. The data from the portable stations alone do not exclude the possibility of a strike-slip solution. However, first motion data from more distant permanent stations for the main shock and some aftershocks exclude the strike-slip possibility.

The consistency of the fault plane solution also indicates that the chosen velocity structure was satisfactory and that the hypocentral depths which range between 3 and 12 km (mostly clustered near 7 km) are reasonable. However, an examination of the depth distribution of aftershocks does not provide any guide to which of the nodal planes in the fault plane solution is the orientation of the fault plane. The scatter suggests that more than one fault plane was involved in the aftershock sequence.

Using the relations described by Kanamori and Anderson⁴ the magnitude of the earthquake can be converted to an approximate moment of 8×10^{23} dyn cm. Using a rigidity modulus of 3×10^{11} dyn cm⁻² and assuming a typical displacement to fault diameter ratio of 10^{-4} (a stress drop of 30 bar) the earthquake source could have been an average of 30 cm of slip on a plane 3.5 km in diameter. The aftershock distribution which has dimensions not much greater than 3.5 km independently supports this rough estimate.

Using a dislocation model⁵ the surface displacements for the earthquake can be calculated from the foregoing results. An uplift of 2 cm is expected approximately above the epicentre

and the area over which displacement greater than 0.5 cm is predicted is shown in Fig. 3. A first-order levelling line crosses the region⁶ and displacements on this line should be observable.

No direct guide to the nature of the source is provided from the geology of the area. In the region of the aftershocks the surface is covered by alluvium generally not more than a few tens of metres thick. Beneath this is some 600 m of Triassic sandstone with shales (perhaps with conglomerates at the base) and this in turn overlies 800–1,600 m of Carboniferous limestones interbedded with sandstones. The Carboniferous rests unconformably on Upper Silurian sediments (greywakes) folded and probably tilted near to vertical. This sequence is thought to extend to a depth of around 12 km.

All hypocentral locations lie within the Upper Silurian which is extensively faulted where it is exposed. Recent work has drawn attention to a major suture in these formations (the lapetus suture) thought to pass through the region of the earthquake^{7,8}. It is possible that the Carlisle earthquake is a reactivation of planes of weakness broadly associated with this suture.

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Ocean bottom seismograph measurements in the Central Aleutians

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Bathymetric profiles of most oceanic trench regions show a well-defined outer rise seawards of the trench with numerous normal faults between the trench axis and the crest of the outer rise. Several investigators (see ref. 1 for review) have shown that the gross bathymetric features can be modelled quite well if they result from a simple mechanical bending of the subducting lithospheric plate. These results have encouraged searches for seismic evidence of the bending stresses predicted by the models. However, the available teleseismic studies² do not determine precisely the relative depths of events on the outer rise, or the precise locations of events relative to the trench. In 1979 we successfully deployed nine ocean-bottom seismometers³ (OBS) which operated for 6 weeks on the outer trench slope in the Central Aleutians between the trench axis and the crest of the outer rise (Fig. 1). The locations, depths and focal mechanisms of the earthquakes located by this network all strongly support the bending hypothesis. In particular, we observed a band of seismicity on the outer trench slope extending ~60 km seaward of the trench axis (Fig. 1). The shallow events (Fig. 2) exhibit normal faulting (Fig. 3) and suggest tension in the upper part of the plate, while the deeper events (Fig. 2) exhibit thrusting mechanisms and suggest compression (Fig. 3). We suggest here that low magnitude seismic activity may be a common feature associated with the well-known normal faulting on the outer trench slope.

The 1979 OBS network located about 100 earthquakes (Fig. 1), including 30 events seaward of the trench axis. Eight of the OBS stations were deployed in an array (Fig. 1) of interlocking triangles with a station spacing of ~30 km. These eight stations covered a rectangular area ~40 km wide, extending from the trench axis to ~60 km seaward of the trench axis. A ninth station (Fig. 1) was deployed ~120 km seaward of the trench axis. No events were located more than 60 km seaward of the trench axis, and (S-P) times observed at the seawardmost station suggest that no such events occurred.

Nineteen of these events were located within about 20 km of the array, and 11 should have especially well determined depths, as they were observed at four or more stations with at least some observations of both P and S phases. Of these 11 events, the depths of seven were shallower than 13 km below sea level, or within about 6 km of the ocean floor, and the remaining four events were located with depths between 30 and 50 km below sea level. None of these earthquakes were located between depths of 13 and 30 km. The station spacing of 30 km allows the determination of relative depths with a precision of 10–15 km for events within or near the station network. Although location using alternate velocity models alters the depths determined for the events, the group of seven events shallower than 13 km remained distinctly shallower than the group of four events deeper than 30 km when these events were located using several different velocity models.

Previously, very little seismic activity has been reported seaward of the trench axis near the site of the present study. Near the trench only one teleseismic event with a tensional focal mechanism has been reported within ~400 km of our network^{2,4}, the event of 22 February 1958, with magnitude

6.75. No events were located seaward of the trench by a network of eight OBS stations that operated on the inner trench wall for 6 weeks in 1978 (Fig. 1). In addition, none of the events located seaward of the trench by the 1979 OBS network were located by the Adak network of seven land stations (Fig. 1) on the volcanic islands ~140 km north of the trench. In this region only 10 events, all with magnitude between 3.0 and 4.9, have been located by the Adak land network⁵ since its installation in 1974. Apparently events seaward of the trench axis are seldom observed by stations landward of the trench either because phases from them are attenuated by structures in or near the trench, or else simply because most of the events are quite small. However, our data show that events with magnitudes of 2.0 and smaller do occur quite regularly in the zone seaward of the trench axis.

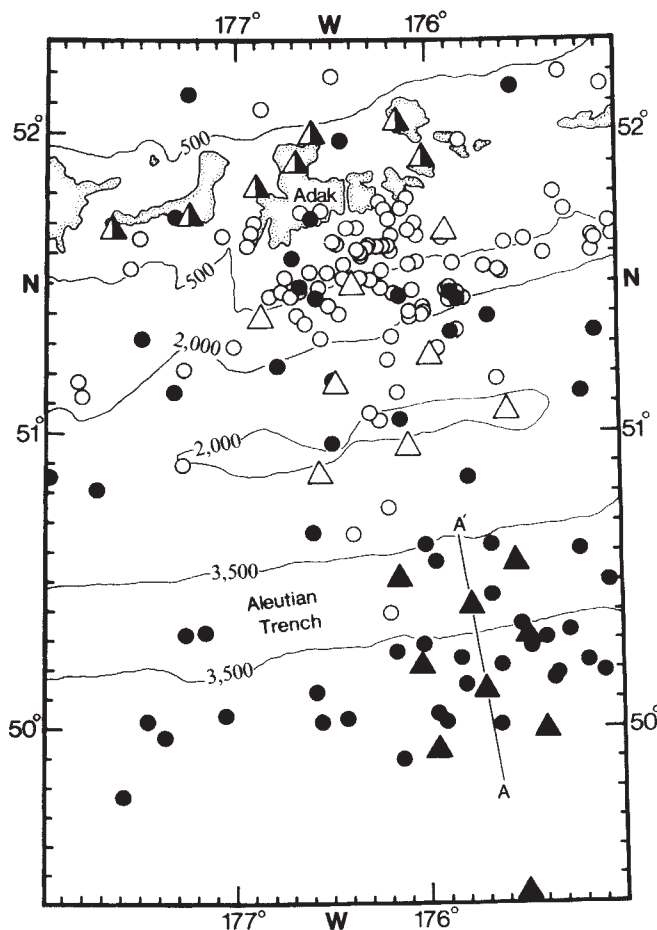


Fig. 1 Location of OBS and land stations in the Central Aleutians, and events located by the 1978 and 1979 OBS network; $\blacktriangle$, 1979 OBS station; $\triangle$, 1978 OBS station; $\blacktriangle$, land station; $\bullet$, earthquake located by 1979 OBS stations; $\circ$, earthquake located by 1978 OBS stations. Bathymetric contours are in fathoms. These events were located by using a program written by C.F. and a layered crustal structure similar to that used by Engdahl⁵. The line between A and A' shows the location of the cross-section in Fig. 2.

With one exception, all the clear first motions of the events within our net were compressions (Fig. 3). For the shallow events, these compressional observations all lie near the edge of the projection of the focal sphere. This does not constrain the focal planes, but for a double couple mechanism, the observations are consistent only with normal faulting. For the deeper events, all the first motions lie in the centre of the projection of the focal sphere. As before, these observations do

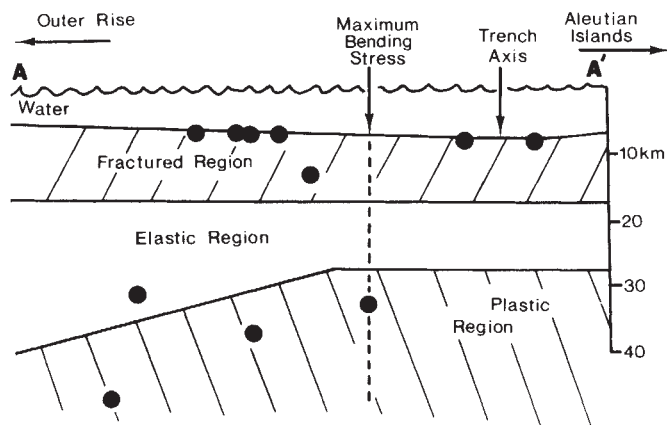


Fig. 2 Vertical cross-section perpendicular to strike of Aleutian Trench (Fig. 1) showing depths of 11 especially well-recorded earthquakes (●) that occurred within or close to the 1979 OBS network (no vertical exaggeration). Note that the Pacific plate extends to the left of the figure, and that it becomes subducted to the right of the figure beneath the Aleutian Islands. The depth chosen for each of these events is that of the minimum weighted r.m.s. residual on plots of r.m.s. residual versus depth. The location of the zone of maximum bending stress as well as the extent of the fractured, elastic and plastic regions were determined by Caldwell⁷ from a study of the bathymetry in the Central Aleutians. For a model with a horizontal compressive stress of 5 kbar, he found that the effective elastic thickness of the lithosphere was ~55 km about 120 km seaward of the trench axis, but that the bending causes the elastic portion of the lithosphere to thin to ~10 km near where the maximum bending stress occurs 18 km seaward of the trench axis. Beneath the elastic zone, the compressional stresses exceed the elastic yield stress, producing earthquakes with compressional (thrusting) mechanisms below a depth of ~25 km. The upper 10–15 km of the lithosphere is a region of little strength^{2,8}, where extensional fractures produce normal faulting events.

not constrain the focal planes, but in this case are consistent only with a thrusting mechanism.

These observations are consistent with normal faulting mechanisms in a shallow zone of extension a few km deep, overlying a quiet zone extending to a depth of ~30 km, which in turn overlies a zone of compression between 30 and 50 km which produces thrusting events. These results are quantitatively consistent with an elastic-plastic model⁶ of the lithosphere with parameters determined solely from the

bathymetry in the Central Aleutians⁷ (Fig. 2). The extremely shallow events with tensional mechanisms are produced by extensional failure in the weak upper portion of the bending plate, while the deeper events occur where the compressional bending stresses exceed the yield stresses in the lower portion of the plate. From our data, the presence of seismicity seaward of the trench axis (Fig. 1) suggests that the yield stress is exceeded to at least 60 km from the trench axis.

Although we have determined reliable depths for only a few events, the present study provides the strongest seismic evidence available suggesting that plate bending occurs on the outer trench slope. Previously, earthquakes whose (S-P) times indicated they occurred seaward of the trench have been detected by an isolated OBS station on the outer trench slope⁹, but this station could not locate the events. The uncertainty in the locations of our located events (Fig. 2) is considerably smaller than is possible for teleseismically located events, although our results are generally consistent with teleseismic observations of events seaward of the trench in several island arcs^{2,10}.

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Northern Hemisphere sea-ice and glacial development in the late Cenozoic

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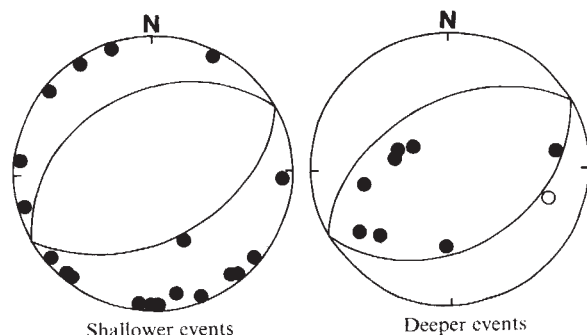


Fig. 3 Composite first motions for the seven shallowest events and the four deeper events that appear in the cross-section (Fig. 2). Compressions (●) and dilatations (○) are plotted on an equal area lower hemisphere projection. The focal planes shown are not constrained by the available first motions, but show clearly that the shallow events are consistent with tensional stresses oriented normal to the trench axis, whereas the deeper events are consistent with compressional stresses oriented normal to the trench axis.

Evidence for the initiation of glacial ice-rafting, and for the development of sea-ice cover has been found in sediment cores from the central Arctic Ocean spanning early Pliocene (~4.5 Myr BP) to Recent time. The glacial evidence reported here includes ice-rafted quartz grains, exhibiting glacially formed surface features, and benthic foraminifera endemic to continental shelves in core sediments from the Alpha Rise. The oldest evidence of ice-rafting occurs in sediments palaeomagnetically dated at ~4.5 Myr BP. Variations in sediment facies indicate varying climatic regimes, and degrees of icecover in the Arctic Ocean since early Pliocene time.

Planktonic foraminiferal fauna compositions and surface features of quartz sand grain in sedimentary cores recovered from drifting ice platforms in the Arctic Ocean from the crest and upper flank provinces of the Alpha Cordillera (Table 1) have been analysed to determine the history of sea ice

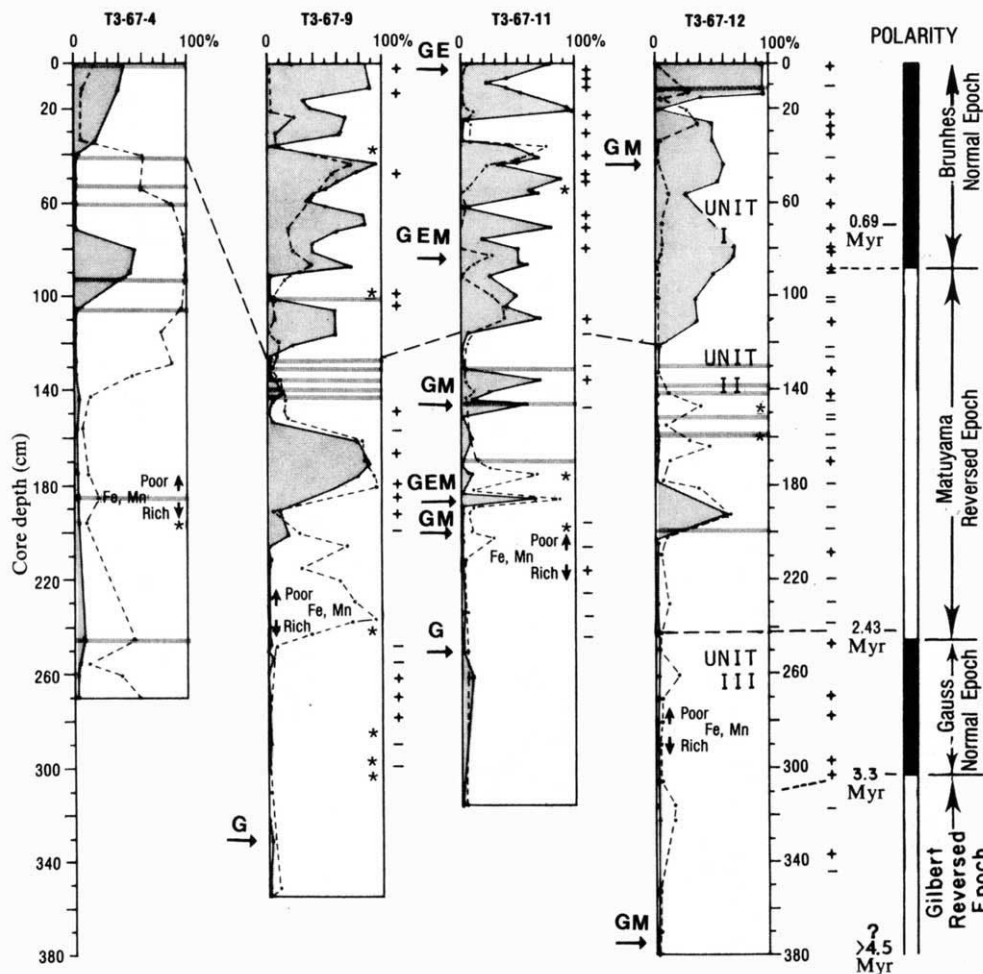


Fig. 1 Palaeomagnetic time scale¹ (right) and percentage of microfauna in coarse fraction (>62 μm) in cores T3-67-4, -9, -11, and -12. Shaded areas, microfauna; white areas, clastic particles. Dashed line and dots represents the percentage of the total planktonic foraminiferal population composed of *G. quinqueloba* and *G. egelida*. Dark horizontal bands are zones in which benthic foraminifera constitute 10% of the total fauna. *, low latitude planktonic foraminifera; +, -, magnetic polarity determinations^{1,11-18}. Dashed lines between cores show age and unit correlations based on palaeomagnetism and lithology. Arrows indicate levels from the cores where quartz sand grains were analysed, and the letters on top of arrows indicate environmental determinations: G, glacial surface features; M, marine (subaqueous abrasion) features; E, aeolian features^{3,4}. Figure modified after ref. 2.

development, iceberg production and polar ice-cap growth of the surrounding continents. Magnetic stratigraphy of these cores¹ and radiometric dates, together with biostratigraphic and lithological correlations (Fig. 1), provide age control points since the early Pliocene². N. D. Opdyke (personal communication) has suggested that core T3-67-12 contains the Gauss/Gilbert boundary at a depth of about 310 cm, and that the bottom of the core is between 4.5 and 5 Myr old. R. Z. Poore (personal communication) thinks that the bottom of this core is still in Gauss sediments with a maximum age of ~3.0 Myr. Opdyke's interpretation is shown in Fig. 1. Within this time interval, we have recognized three major oceanic sediment regimes in the Arctic, here represented as climatic units. The earliest, Unit III, contains sediments deposited between ~4.5 and 2.5 Myr (ref. 1) and consist of manganese-iron micronodule-bearing red clays. Rock fragments and mineral grains of ice-rafted origin were identified from three samples from this unit (Fig. 1). As this represents perhaps the oldest described evidence of Arctic Ocean ice-rafting, confirmation of the glacial (ice-rafted) origin of these sediments was sought by scanning electron microscopic examination of surface features on sand grains for environmental determinations^{3,4}. Quartz sand grains from Unit III from sediments ~4.5 Myr BP (ref. 1) exhibit surface features (Fig. 2) consistent with a cycle of glacial abrasion before their transport to this region from the surrounding shelves and/or continents. Quartz sand grains were selected from the >62 μm washed fraction from representative sections of each climatic unit. The samples examined are indicated by arrows on Fig. 1. Two samples, not illustrated, from Drill Station A-4 (Table 1) which contains sediments of Brunhes age belonging to Unit I were also examined and found to contain glacial and glacial marine features. About 50 grains were

selected randomly from each sample, to represent the range of sizes and shapes present. The grains were petrographically examined to ensure that they were uniaxial quartz, and then examined by scanning electron microscopy to determine the occurrence and distribution of environmentally diagnostic surface features as described in refs 3, 4. A more thorough presentation of these data will be published elsewhere. Grains also show evidence of subaqueous abrasion (Fig. 2) indicative of either current or wave action after the glacial abrasion. The grains examined from these samples correspond to the 'glacial' and 'glacial marine' categories^{3,4}. Sand grains such as these could be derived from glacial deposits from the continental regions surrounding the Arctic Ocean, as well as from shelves, and transported by icebergs, shelf ice, or sea ice hundreds of kilometres to their present site.

The fauna in sediments from Unit III is sparse, constituting <1% of coarse fraction (>62 μm, Fig. 2). The planktonic foraminifera are dominated by the robust, polar, left-coiling *Neoglobobulimina pachyderma* complex, some of which are corroded. Shell fragments are abundant, and the solution susceptible tests of juvenile specimens are rare in this unit.

Scattered occurrences of low latitude planktonic foraminifera in this unit as well as in the 'foraminifera-poor' layers of Units II and I have been reported elsewhere^{2,5} (Figs 1, 3). Their presence was suggested to be due to transport by ocean currents from the Atlantic and Pacific during periods of more active water exchange between the Arctic and adjacent oceans⁶ or to transport by shelf ice (Y. H. unpublished data). Barents Sea data indicate that shells of planktonic organisms carried from low latitudes by the warm Norway Current and deposited on the floor of the Barents Shelf may have been incorporated into grounded shelf ice. Subsequently, during periods of calving and drift over the Arctic, the incorporated

debris was dropped to the sea floor. Benthic foraminifera are represented by deep-water (solution-resistant) agglutinated forms and robust, calcareous elements^{2,5}. Rare, shallow-water elements are also present, indicating transport by icebergs and/or islands from shelf areas^{2,5}.

The Unit III/II boundary is defined by simultaneous lithological and faunal changes, and coincides very roughly with the Gauss/Matuyama boundary (Fig. 1). The change is from the red clays in Unit III to tan silts in Unit II, which contain more abundant coarse ice-rafted sediments. Ice-rafted quartz sand grains from this unit differ from those of Unit III not only in abundance, but also in their surface features. Grains from Unit II show a greater percentage of subaqueous abrasion, overgrowths, and also aeolian features in comparison to features of glacial origin (Fig. 2). The aeolian/subaqueous/glacial combination are typical of periglacial environments presently found in Alaska³.

Planktonic foraminifera in Unit II are dominated by the extant subpolar species *Globigerina egelida* and *Globigerina quinqueloba*. These two solution susceptible species constitute up to 99% of the fauna in cores raised from depths shallower than ~2,400 m; however, their relative abundance decreases considerably in deeper water sediments^{2,5} (Figs 1 and 3). The right-coiling subpolar *N. pachyderma* complex attains highest frequencies in this unit's sediments⁵ (Fig. 3).

The benthic foraminifera have both deep- and shallow-water representatives. Among the latter, species belonging to *Elphidium*, endemic to continental shelves, are abundantly represented, constituting up to 50% of the benthic elements (Fig. 3). Deep-water species are varied, both calcareous and arenaceous elements being common⁵.

The Unit II/I boundary is again defined by both faunal and lithological changes, and occurs near the Brunhes/Matuyama magnetic boundary² (Fig. 1). Brunhes sediments, representing the last major climatic regime, are composed of alternating foraminifera-rich and foraminifera-poor layers. The former represent conditions similar to those prevailing today (perennial sea-ice cover)⁵; the latter perhaps short, milder, lower salinity intervals, comparable to those of the Matuyama epoch. The planktonic fauna is dominated by the polar left-coiling *N. pachyderma* complex; however, *G. quinqueloba*, capable of withstanding low salinities (Y.H. unpublished data), attains high frequencies near several of the foraminifera-rich/foraminifera-poor boundaries; benthic foraminifera are varied and ice-rafted debris is scattered throughout Brunhes sediments (Figs 1 and 3).

Two samples from the foraminifera-rich zones of Unit I show surface features indicative of glacial marine and aeolian periglacial origin (Fig. 2), while grains from the foraminifera-poor zones show typical glacial surface features similar to those from Unit II Matuyama sediments.

The differences between quartz grains from the three units can be summarized as follows: samples from Unit III contain fewer and smaller (<250 µm) grains and higher percentage of rounded aeolian and subaqueously abraded grains. Grains with glacial evidence also show superimposed features of subaqueous and aeolian origin. Samples from Unit II contain a greater number of large (>250 µm) and angular grains, exhibiting a mixture of aeolian and subaqueously abraded

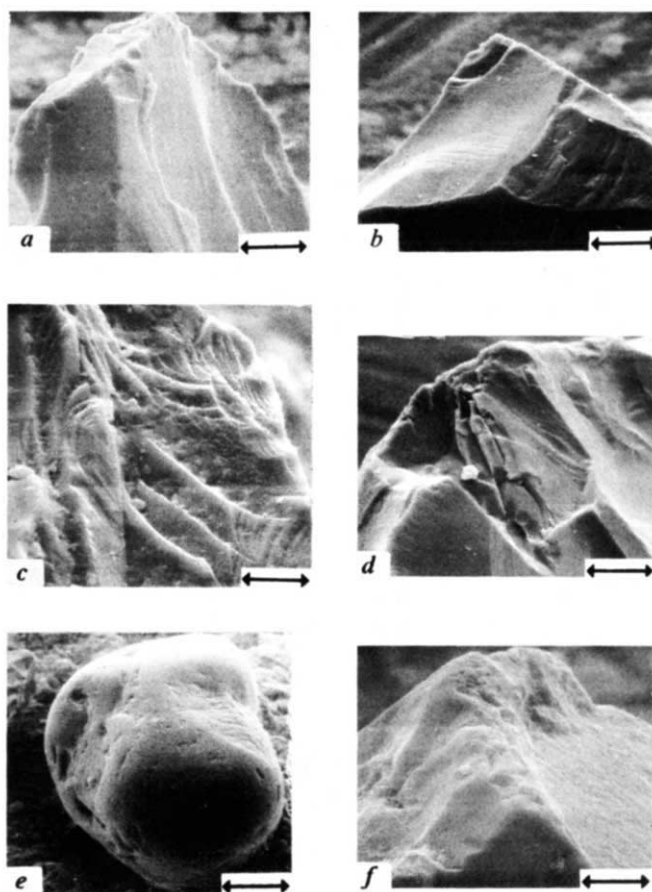


Fig. 2 Scanning electron micrographs of quartz sand grains from Arctic Ocean cores shown in Fig. 1. *a*, Sand grain from core T3-67-9, 330 cm depth (Unit III). Showing sharp angular outline, step shaped fractures and conchoidal fractures; typical of grains subject to glacial action, scale bar, 20 µm. *b*, Edge of quartz grain from core T3-67-12, 375 cm depth (Unit III), showing conchoidal fractures, semi-parallel steps, striations, and other features shown in *a*, scale bar, 20 µm. *c*, Sand grain from core T3-67-11, 85 cm depth (Unit I) showing high relief, large breakage blocks and conchoidal fractures typical of grains of glacial origin, scale bar, 10 µm. *d*, Grain from core T3-67-11, 188 cm depth (Unit II) showing typical glacial surface features over a larger area of the grain, scale bar, 20 µm. *e*, Grain from core T3-67-11, 188 cm depth (Unit II) showing abrasion features and rounded outline typical of grains with a history of marine-subaqueous transport, scale bar, 200 µm. *f*, Grain from core T3-67-9, 330 cm depth (Unit III) showing glacial features modified by aeolian features along right-hand margin, and on centre ridge, scale bar, 20 µm.

features. Unit I samples contain many large angular grains of glacial origin, with relatively few grains of mixed transport cycles.

The number of quartz grains of glacial increases in progressively younger sediments, while there is a decrease in the number of grains exhibiting features of mixed origins. In Brunhes sediments, however, there seems to be periods during which sediment transport conditions similar to those of the Matuyama prevailed.

There is relatively little information on pre-Pleistocene sediments from the Arctic Ocean. Ling *et al.*⁷ reported evidence for a warm, ice-free Arctic Ocean during Eocene and later times. A warm, temperate Arctic climate for the Palaeogene is discussed by Frakes and Kemp⁸. The evidence of vigorous vertical mixing and relatively high biological production of the Arctic surface water preclude the existence of perennial ice cover between the late Miocene and the late Pliocene^{2,5,9}.

About 2.5 Myr ago a dramatic change in the structure of the Arctic Ocean water masses occurred, including the inception of density stratification. A stable, low-salinity, surface-water mass,

Table 1 Locations, depths and lengths of cores

Core	Lat.	Long.	Depth (m)	Length (cm)
D.St.A.2	83°52'N	168°12'W	1,521	206
D.St.A.4	84°21'N	168°49'W	2,041	116
T3-67-3	79°11'N	175°09'W	2,285	380
T3-67-4	79°22.7'N	174°46'W	1,760	272
T3-67-9	79°37.9'N	172°07'W	2,237	356
T3-67-11	79°34.9'N	172°30'W	2,810	250
T3-67-12	80°21.9'N	173°33'W	2,867	374

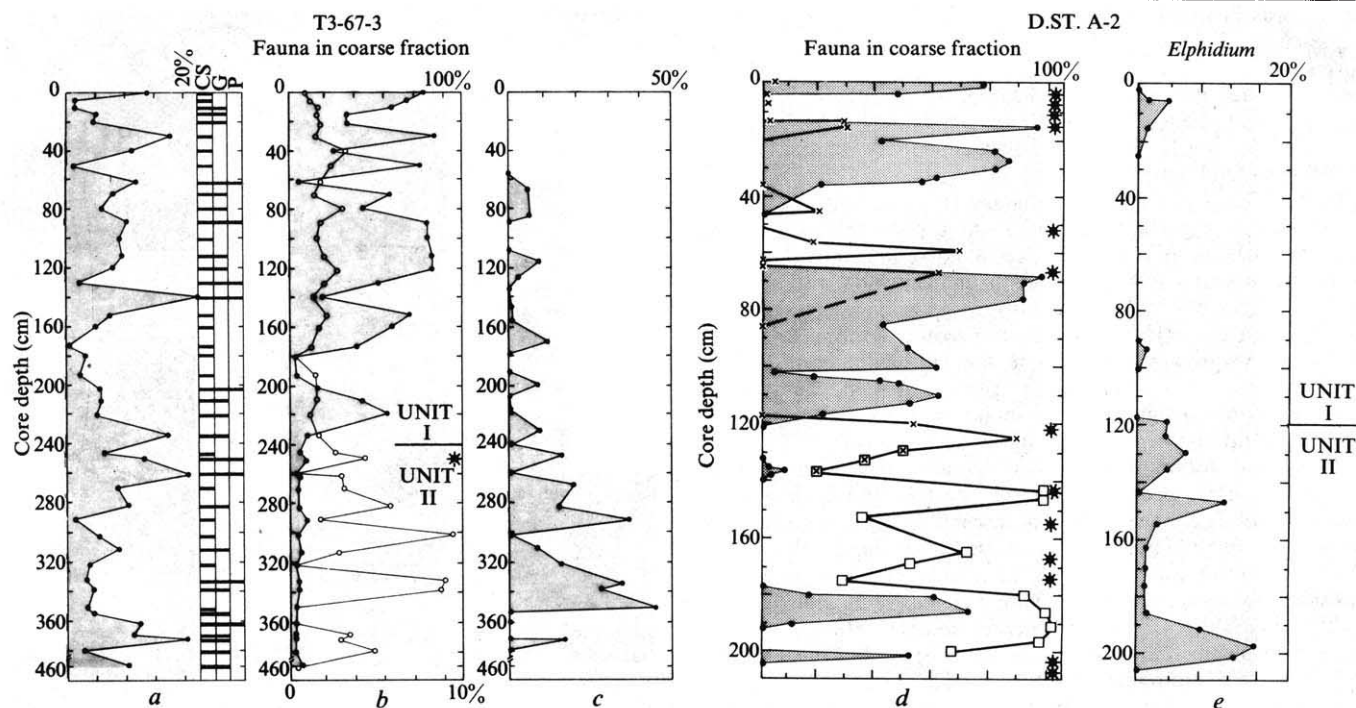


Fig. 3 Core T3-67-3. *a*, Percentage of coarse fraction ($>62\mu\text{m}$). CS, coarse sand; G, granules; P, pebbles. *b*, Percentage of microfauna in the coarse fraction ($>62\mu\text{m}$). Shaded areas represent the microfauna, white areas the clastics; values are indicated at the top of the diagram. $\circ$, percentage of dextral *G. pachyderma* out of the total *G. pachyderma* population; values are indicated on the abscissa at the bottom of the diagram. *, low latitude planktonic foraminifera. *c*, Percentage of *Elphidium* spp. out of the total benthic foraminiferal fauna. (Modified after ref. 2.) *d*, Percentages of microfauna in the coarse fraction ($>62\mu\text{m}$). D. St. A2. Shaded areas represent the microfauna, white areas the clastic material. $\times$, percentages of *G. quinqueloba* out of the total planktonic foraminiferal fauna. $\square$, percentages of *G. egelida* of the total planktonic foraminiferal fauna. *, low latitude planktonic Foraminifera. *e*, Percentages of *Elphidium* spp. a shallow-water genus out of the total benthic foraminiferal fauna. (Modified after ref. 2.) Drill Station A2.

essential for sea ice formation, was developed at this time^{2,5}. Whether temperatures were sufficiently low for pack ice to form, even seasonally, cannot be ascertained for certain with the present data. While most data suggest that global Matuyama temperatures were higher than Brunhes temperatures (see refs 11–17), Kennett¹⁸ interprets his results to indicate the opposite. It can be assumed, however, that given the stratification, sea-ice cover was present during the winter. A major climatic threshold was crossed ~ 0.7 Myr ago, when, as a result of continued global cooling, perennial sea-ice cover developed over the Arctic Ocean^{2,5}.

Clark, however, has stated that the Arctic Ocean has been covered continuously with perennial ice from the middle Cenozoic to the present and that the ice cover was much thicker during the deposition of the 'foraminifera-poor beds' than today¹⁹. At present, the ice attains thicknesses of 3–4 m at the end of winter, decreasing to ~ 2 –3 m in summer²⁰. Field observations and theoretical calculations indicate that sea ice reaches an equilibrium thickness at ~ 4 m (ref. 21). Furthermore, evidence exists for much drier climates, with reduced snowfall during glacial episodes in the north polar region. This is a direct consequence of increased continentality caused by broadening of coastal plains due to lowered sea level⁹, and reduction of moisture available to the atmosphere when ice covered extensive land and ocean areas.

Recently discovered calcareous nannofossils in Unit III sediments as well as in 'foraminifera-poor zones' and in transition sediments between foraminifera-poor and foraminifera-rich zones also preclude the existence of perennial ice cover during the deposition of these sediments²² and further supports the interpretation of Herman^{2,5} concerning the evolution Arctic oceanic-climate during the past 5 Myr.

Glacial-marine (ice-rafted) sediment was previously reported from the North Pacific Ocean by Krinsley²³ who found glacial surface features on quartz grains of late Pliocene age from DSDP site 178 (Alaskan Abyssal Plain). Other

investigators^{24–26} found evidence of ice-rafting in the North Pacific, Bering Sea, and the Norwegian–Greenland Seas in sediments of middle Pliocene age (~ 2.4 –3 Myr old), which is consistent with the oxygen isotopic evidence for major development of Northern Hemisphere glaciers about this time²⁷. One core raised at site 344 ($76^{\circ}08.98'N$; $07^{\circ}52.52'E$) contains glacial marine sediments throughout the Pliocene. It is not certain whether the entire Pliocene was recovered nor what processes of sedimentation were responsible for the deposition of the glacial marine debris: "ice rafting, turbidity currents or sedimentary suspension in water" has been suggested.

Occurrence of ice-rafted quartz sand grains and shallow-water benthic foraminifers endemic to continental shelves on the crest and upper flank provinces of the Alpha Rise in sediments deposited ~ 4.5 Myr ago² firmly establishes that high latitude Northern Hemisphere mountain glaciers developed and perhaps extended to sea level at least as early as the early Pliocene. In addition, the variations in sediment types, microfaunal and floral compositions, and abundance of ice-rafted and glacially produced detritus, all indicate the history of development of ice cover in the Arctic Ocean. Corroboration by similar data from other Arctic Ocean sites would make this date highly significant with respect to our understanding of the chronology of Northern Hemisphere glaciations and the eventual onset of the Pleistocene glacial/interglacial climatic cycles. Oxygen isotope analysis of calcareous foraminifera and nannoplankton from these cores is planned to correlate these sediment sequences with isotopic stratigraphies established elsewhere in the world's oceans.

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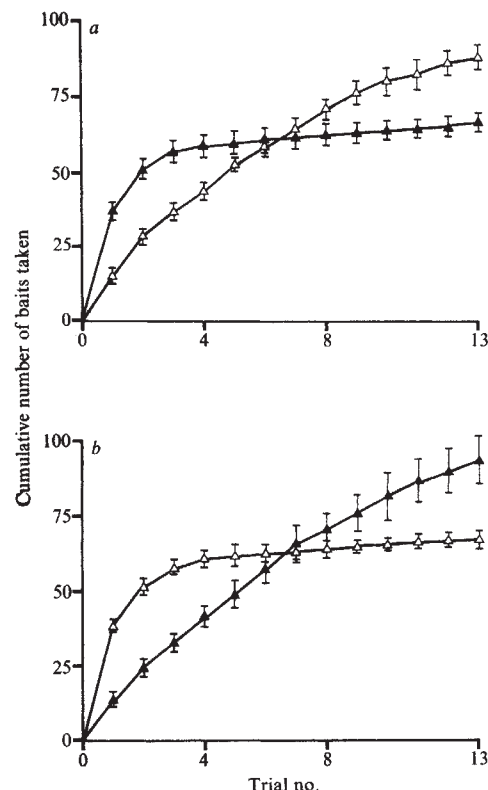


Fig. 1 Cumulative number of baits taken in successive trials. Experimental series: *a*, background green; *b*, background blue. Closed symbols represent experiments with blue food and open symbols green food. 95% confidence limits are shown.

Why are distasteful prey not cryptic?

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Bright coloration among unpalatable prey is common¹. Turner² has suggested that, as with Fisher's explanation for the evolution of distastefulness itself³, such aposematic coloration has been favoured by kin selection. Distasteful aposematic prey tend to live in kin groups and predators sampling from such groups will learn to avoid conspicuous prey more readily than cryptic prey and, as a consequence, fewer members of conspicuous kin groups are preyed upon^{2,4-6}. We know of no clear evidence that vertebrate predators take fewer trials when learning to avoid conspicuous rather than cryptic distasteful prey^{6,7}. Here we report results from a series of experiments that provide such evidence.

For 3 days post-hatch, male Warren Sex-Link chicks were fed on dyed blue and green chick crumbs of equal size, hue and texture, that were presented on a grey background against which we could detect no selective predation⁸. On day 4, individual chicks were placed in a holding box for 1 min before being released through a sliding door into a testing arena (80 × 60 cm) with a hardboard painted floor. The floor was interchangeably blue or green and on it were scattered 1,000 chick crumbs which had been made distasteful (by including 4 g quinine sulphate and 2 g mustard powder per 100 ml of dye solution). At this density the crumbs do not act as background to each other and selective predation by the chicks is similar to that at lower densities⁹. The crumbs were all the same colour, either blue or green. Individual chicks were allowed to feed for 2 min and were retested every hour for 13 h, that being the number of trials after which each chick had refused food on at least one occasion. Details of housing, lighting conditions, food preparation, the testing arena and feeding before experimentation, are as described elsewhere⁷. There were four treatments—floor and food blue, floor

and food green, floor blue and food green, floor green and food blue—with nine chicks assigned to each.

The number of crumbs taken during the first (n_1) and subsequent trials (n_s), together with the number of trials before each chick refused food for the first time are shown in Table 1. In the first trial, more blue than green baits were taken when each was presented on a green background (Mann-Whitney $U = 0$, $P < 0.01$). Similarly, during the first trial more green were taken than blue when each was presented on a blue background ($U = 0$, $P < 0.01$). This we interpret as demonstrating crypticity of the crumbs against matching backgrounds. However, by the final trial the situation had changed: more green baits than blue had been taken ($n_1 + n_s$) against the green background ($U = 2$, $P < 0.01$), and more blue baits than green had been taken on the blue background ($U = 0$, $P < 0.01$). From the results shown in Fig. 1 it is apparent that the diverges to the two treatments in each experiment were still diverging when testing was stopped.

These experiments indicate that chicks learn to avoid conspicuous more readily than cryptic distasteful prey. One or both of two mechanisms could be operating. First, the

Table 1 The number of crumbs taken during the first trial (n_1), the total number taken during the 12 subsequent trials (n_s), and the number of trials before each of the 36 chicks was tested and refused to feed for the first time (r)

Crumb colour Background colour	Blue			Green			Blue			Green		
	n_1	n_s	r	n_1	n_s	r	n_1	n_s	r	n_1	n_s	r
Blue	15	67	9	41	29	6	45	30	4	10	60	9
Green	18	82	11	35	36	4	39	26	5	16	85	11
Blue	11	68	10	33	25	4	40	20	3	9	96	10
Green	20	65	10	40	26	3	35	36	5	20	84	11
Blue	17	72	9	29	33	4	36	28	4	14	73	7
Green	16	70	10	44	25	3	35	33	6	10	87	9
Blue	13	72	10	38	26	5	38	29	5	15	89	11
Green	9	81	12	36	35	4	42	27	5	17	79	10
Blue	19	77	10	39	31	3	37	28	6	13	66	9

appearance of conspicuous prey *per se* is learned more readily than that of cryptic prey. Second, eating large numbers of prey in a short time is a more powerful reinforcer than eating even greater numbers of prey over a longer time period. Either mechanism provides a proximate explanation for the functional hypothesis. Note that in these trials the chicks were not offered a food choice and that, therefore, the experiments do not mimic the evolution of aposematic coloration⁷. Whether the effect described here is large enough to favour the evolution of conspicuous coloration among unpalatable prey depends on various behavioural parameters, such as prey group size and the availability of other foods to the predator, in addition to the degree of unpalatability or toxicity of the prey.

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Dopaminergic A10 neurones are involved in cognitive functions

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Delayed response tasks are widely considered to be particularly sensitive in demonstrating cognitive deficits after lesions of the prefrontal cortex in all species of mammals¹. In rodents, these deficits are usually tested in a T-maze and are more pronounced after lesion of the anterior part of the striatum than after prefrontal cortex ablation². In effect, the striatum is capable of assuming cortical functions either when the prefrontal cortex is poorly developed as in the lower mammals³ or immature as in the early life of primates⁴. The prefrontal cortex and anterior striatum—the so-called prefrontal system⁵—are functionally and anatomically related. The prefrontal area specifically involved in delayed alternation⁶ receives projections from the mediodorsal thalamic nucleus (MD)⁷ and sends axons to the anterior striatum as well as to the MD^{3,7}. Recently, a detailed anatomical study of the connections of the prefrontal system revealed the existence of important projections from the mesencephalic dopaminergic A10 neurones and the mediodorsal nucleus that converge on the prefrontal cortex⁸. Axons of the A10 system also project to the anterior part of the striatum^{9,10}. The influence of dopaminergic A10 neurones on frontal lobe functions remains obscure, but some observations concerning the behavioural, pharmacological and biochemical effects of neuroleptics and psychostimulants have supported the idea that these dopaminergic neurones could have a role in the pathogenesis of schizophrenia^{11–13}. Furthermore, the major symptoms observed in schizophrenic patients such as cognitive and attention disturbances have been attributed to a dysfunction

of both prefrontal cortex and striatum^{14–16}. Thus, in addition to their anatomical connections, the dopaminergic A10 neurones may be functionally related to the prefrontal cortex and striatum. These data encouraged us to investigate whether A10 neurones are functionally related to the prefrontal system and in particular if they are involved in cognitive processes measured in a delayed alternation task. We demonstrate here that selective lesions of dopaminergic A10 neurones in the rat produce a severe and specific impairment in retention of delayed alternation. On anatomical and behavioural grounds the dopaminergic A10 system could therefore be considered as a rather better indicator of frontal system function than the mediodorsal thalamic nucleus.

Fifteen male Sprague-Dawley rats (300–350 g) received, under ketamine anaesthesia (150 mg kg⁻¹ intraperitoneally (i.p.)), bilateral stainless steel guide cannulae (0.65 mm) aimed 6.5 mm above the A10 cell bodies region. Stereotaxic coordinates were: 4 mm posterior to the bregma, 0.5 mm lateral to the midline and 2.2 mm below skull surface. Fifteen days after surgery, the delayed alternation experiment began according to the procedure of Wikmark *et al.*². Briefly, animals deprived of food for 12 h were trained to enter alternately each arm of a T-maze for food with a fixed time between trials. Twenty-one trials were carried out daily until each rat made five errors or less in 3 consecutive days (91.66% of correct responses in 60 trials).

After the learning period, the animals were divided into control (*c* = 6) and experimental (*e* = 9) groups receiving vehicle or 6-hydroxydopamine (6-OHDA, 2 µg µl⁻¹) injections respectively. The injections were made in unanaesthetized animals by lowering bilaterally an injection needle (0.30 mm) through the guide cannulae to the region of A10 cells (8.7 mm below the skull surface). A second injection was performed in similar conditions 3 d later. After another 10 d, the animals were tested for retention of delayed alternation according to the same paradigm as that used in the learning sessions. Training continued until the pre-injection criterion was reached.

Post-operative performances and gross behavioural analysis showed that experimental (6-OHDA-A10) animals presented a severe impairment in retention of delayed alternation. As indicated in Table 1, control rats showed no deterioration in performance between pre- and post-operative behavioural testing, as the number of errors was not statistically different. Moreover, the number of trials in retention was only slightly higher than the number of 60 trials imposed to reach the criterion. On the other hand, the 6-OHDA-A10 rats performed badly. In the first 60 post-injection trials, the 6-OHDA-A10 rats made many more errors than the control group (*t* = 7.1; *P* < 0.001). There was no difference in the errors made by the

Table 1 Learning and retention scores of delayed alternation responses

Group (n)	Errors in the last 60 pre-operative and in the first 60 post-operative trials (mean ± s.e.m.)		No. of trials to reach criterion (mean ± s.e.m.)	
	Pre-operative	Post-operative	Pre-operative	Post-operative
Control (6)	4.7 ± 0.3	5.1 ± 0.7	146.6 ± 12.3	70 ± 4.5
6-OHDA-A10 (9)	4.7 ± 0.2	19.6 ± 1.2*	148.9 ± 10	313.3 ± 33.9

There was a significant difference between control and experimental (6-OHDA-A10) groups in the number of errors in the first 60 post-operative trials and the number of post-operative trials needed to reach the criterion (Student's *t*-test, *P* < 0.001). As indicated by the number of errors in the first 60 post-operative trials compared with the last 60 pre-operative trials, the control animals did not show any deterioration of performance. On the contrary, many more post-operative than pre-operative trials were needed by the experimental (6-OHDA-A10) rats to reach the criterion (*paired test, *P* < 0.01).

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Table 2 Learning and retention scores of visual discrimination

Group	(n)	Errors in the first 60 trials (mean $\pm$ s.e.m.)	No. of trials to reach the criterion (mean $\pm$ s.e.m.)	
		Post-operative	Pre-operative	Post-operative
Control	(5)	6.6 $\pm$ 0.5	116 $\pm$ 4	72 $\pm$ 5
6-OHDA-A10	(7)	6.3 $\pm$ 0.4	111.4 $\pm$ 6	71.4 $\pm$ 4

No statistical difference was observed between control and 6-OHDA-A10 lesioned rats. The number of trials for all rats in retention was only slightly greater than the 60 trials imposed to reach the criterion.

6-OHDA-A10 group between the first 60 post-operative trials (32.66%) and the first 60 initial trials in the learning period (32.40%). Post-operatively, the number of trials to reach the criterion was greater in 6-OHDA-A10 rats compared not only with the control group ($t = 5.6$; $P < 0.001$) but also with their own pre-injection level of performance ($t = 5$; $P < 0.001$). Gross behavioural analysis showed that, during the pre-operative learning of the delayed alternation response, the two groups of rats exhibited collateral behaviours such as rearing, stopping and returning before entering the chosen arm of the T-maze. These collateral behaviours were frequently observed at the beginning of acquisition and then disappeared progressively as the number of errors decreased. Post-operatively it was observed that the 6-OHDA-A10 rats demonstrated more collateral behaviours than the control rats. This difference was particularly evident during the 20 trials of the third post-operative session, that is when the control rats nearly reached the criterion (mean of rearings: $e = 2.10$, $c = 0.33$; stops: $e = 4.2$, $c = 2$; returns: $e = 2.20$, $c = 0.67$). According to these results the mean running time from the start box to the goal box of the T-maze was greater in 6-OHDA-A10 rats ($e = 4.5$ s, $c = 2.08$ s; Student's t -test: $t = 6.9$; $P < 0.001$). This running time did not shorten during the following trials, possibly indicating that post-operatively the experimental animals did not really relearn the task but rather developed a new strategy to reach the criterion. These behavioural observations might explain the nature of the deficit observed in the delayed task after lesion of A10 neurones. The experimental animals showed post-operatively more collateral or 'irrelevant' behaviours because they presented attentional deficits manifested by an enhanced distractibility or responsiveness to environmental stimuli. This renders the animals unable to select relevant information. In this respect, it is interesting that the frontal syndrome has also been interpreted in terms of attentional deficits¹⁷.

Whatever the nature of the perturbation observed, the specificity of our results could be criticized. First, considering the fact that the experimental animals reached the food reward more slowly than control animals, the retention impairment could be explained by motivational deficits. To test this hypothesis, animals were trained in a straight alley divided into a start box, a runway and a goal box containing food reward. The running time for deprived animals to reach the goal box was considered as an index of their motivational state. Each animal was made to run daily in 10 consecutive trials over a period of 5 d. At the end of the experiment, it was found that the running time did not differ between the experimental (mean: 6.28 ± 1.31 s) and the control groups (mean: 5.93 ± 1.12 s). Second, it remained important to investigate if the perturbation observed in the delayed alternation task was not the result of more general learning disabilities. In this respect, it has been found that prefrontal lesioned animals have deficits in delayed alternation but not in discrimination tasks¹. To test the specificity of a perturbation of delayed alternation after lesion of A10 neurones we carried out an additional experiment. Twelve naive rats maintained on 90% of *ad libitum* body weight, were trained in a Y-maze of the Lashley type for a visual discrimination task. Food reward signaled by light was randomly placed in the right or left arm of the maze. Twenty trials took place daily until the animals reached the criterion of 90% correct responses (6 errors

Table 3 Dopamine and serotonin contents after 6-OHDA injection ($2 \times 2 \mu\text{g } \mu\text{l}^{-1}$) in the region of dopaminergic A10 cell bodies

Group	(n)	Dopamine (ng per g)			Serotonin (ng per g)	
		Frontal cortex	Nucleus accumbens	Anterior striatum	Frontal cortex	Hippocampus
Control	(6)	126 $\pm$ 10	8,290 $\pm$ 590	8,190 $\pm$ 1,310	539 $\pm$ 62	342 $\pm$ 33
6-OHDA-A10	(9)	39.7 $\pm$ 5.7	1,260 $\pm$ 150	2,230 $\pm$ 670	495 $\pm$ 26	241 $\pm$ 25

Note that 6-OHDA-A10 rats showed a big depletion of dopamine in forebrain projections, whereas serotonin levels were not significantly modified in the frontal cortex and decreased only slightly in the hippocampus. Values are mean $\pm$ s.e.m.

or less in 3 consecutive sessions of 20 trials each). Then, the rats were divided into control ($n = 5$) and experimental ($n = 7$) groups. The control group rats received a saline injection and the experimental group 6-OHDA injections in the A10 cell bodies region, strictly according to the procedure previously used for the delayed alternation test. Fifteen days after the injections the rats were tested for retention of visual discrimination. As indicated in Table 2, the number of trials to reach the criterion and the mean of errors in the first 60 post-operative trials are similar for the two groups of rats.

At the end of the behavioural experiments, animals were decapitated and the brain quickly removed. The posterior part of the brain was kept out for histological examination of injection sites and the anterior part was used for biochemical assays as described previously¹⁸. Table 3 shows the biochemical results. Note that brain dopamine content of 6-OHDA rats was greatly depleted in the forebrain projections of A10 cell bodies such as the frontal cortex (-68.5%), the nucleus accumbens (-84.8%) and the anterior part of the striatum (-72.8%), whereas endogenous serotonin corresponding to raphe projections was not modified in the frontal cortex or only slightly decreased in the hippocampus (-29.5%).

In conclusion, our results suggest that the dopaminergic A10 neurones have a fundamental role in the processes required for the retention of the delayed alternation response. Supporting this view, it has recently been found that 6-OHDA injections in the frontal cortex of the monkey¹⁹ and rat²⁰ or in the striatum of the rat^{20,21} disrupted the delayed alternation response.

Moreover, we have demonstrated the critical influence of brainstem neurones on higher mental functions, such as cognitive processes, which have often been attributed exclusively to the prefrontal cortex. It could be considered that the sensory information reaching the A10 neurones²² and conveyed to the prefrontal cortex and striatum are of particular importance for the normal functioning of the prefrontal system. Careful analysis of these A10-prefrontal system interrelations may be of special interest for future research dealing with cognitive representational functions and some pathophysiological states.

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The role of ventral bundle noradrenergic neurones in sensory components of sexual behaviour and coitus-induced pseudopregnancy

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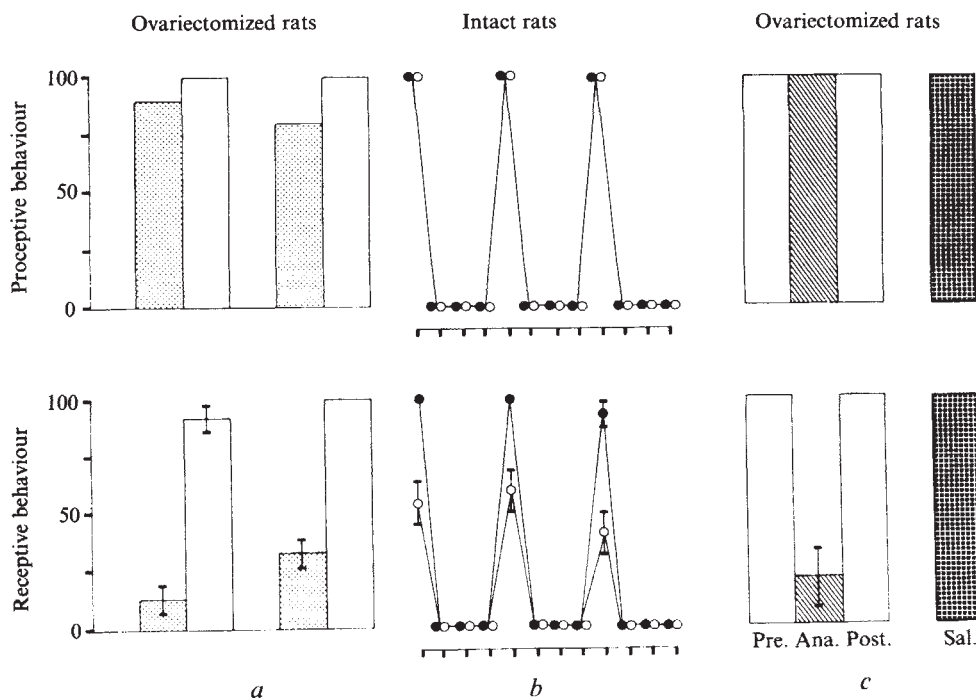
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Interaction of the female rat with the male in standardized laboratory conditions is characterized by two categories of behaviour. The first, known as proceptive behaviour, consists of a series of rapid movements by which she approaches and leaves the male, hopping and darting and wiggling her ears¹. This behaviour, which is a response to visual, olfactory, and probably auditory cues from the male, serves to attract his sexual interest. As soon as the male mounts her, this behaviour is abruptly terminated and is replaced by a second (receptive) pattern¹. The female becomes completely immobile and dorsiflexes her vertebral column to display a lordotic posture, which exposes her vagina and facilitates intromission by the male; this pattern results from tactile stimulation received by the female from the male as he mounts her^{2,3}. Both these behaviours are under endocrine control since the female rat will only show them if she is exposed to the ovarian hormones oestradiol and progesterone⁴. However, proceptivity and receptivity clearly differ with regard to the neural mechanisms responsible for their activation, since different groups of afferent stimuli are required to set them in motion. We now show that a specific part of the ascending systems of noradrenergic neurones in the brain, that is carried in the ventral noradrenergic bundle⁵, is critically involved in the mechanisms by which tactile stimuli elicit receptive, but not proceptive, behaviour in the female rat. The same system is also involved in the process by which genital stimuli activate another neuroendocrine phenomenon, the induction of pseudopregnancy.

The first experiment was carried out on ovariectomized animals injected daily with a low dose of oestradiol monobenzoate (0.2 µg) and with a single injection of progesterone (0.5 mg) between 4 and 6 hours before behavioural testing. Figure 1a shows that animals in which an electrolytic lesion of the ventral noradrenergic bundle had been made (Fig. 2) continued to display proceptive behaviours towards the male. However, in contrast to controls (who showed high levels of receptivity) ventral noradrenergic bundle-lesioned animals displayed greatly reduced levels of lordosis (receptive behaviour). The procedure was then repeated except that instead of an electrolytic lesion, 6-hydroxydopamine (6-OHDA) was injected at the same site. This neurotoxin induces selective degeneration of noradrenergic neurones when placed discretely in the small dose and volume (2 µg base in 0.5 µl Ringer's solution) used here⁶. Figure 1a shows that this resulted in similar behavioural consequences, females continuing to show proceptive behaviour, but only low levels of lordosis when they were mounted by the male. Table 1 shows that both electrolytic lesions (Fig. 2) and those produced by the neurotoxin 6-OHDA (Fig. 2) produced similar neurochemical changes, in that noradrenaline uptake in the hypothalamus was substantially decreased, whereas that in the cerebral cortex (which is supplied by the dorsal noradrenergic pathway) was unaffected. Very little or no nonspecific damage was seen around the site of injection of 6-OHDA and careful histological examination revealed that lemniscal and spinothalamic projections were undamaged. These experiments therefore suggest that the behavioural results that we observed were produced specifically by decreasing the noradrenaline content of the subcortical brain after a ventral noradrenergic bundle lesion.

The second experiment was carried out on intact animals showing regular oestrous cycles. The vagina of each rat was covered with tape to prevent intromissions during sexual interaction and so avoid episodes of pregnancy or pseudopregnancy. Such animals show recurrent periods of active sexual behaviour ('heat') every fourth day. Electrolytic lesions in the ventral noradrenergic bundle similar to those made in the first experiment did not prevent the postoperative occurrence of normal 4-day vaginal oestrous cycles, although these took longer to be re-established after operation than in the sham-operated

Fig. 1 The effects of ventral noradrenergic bundle (VNAB) lesions or procaine on the receptivity and proceptivity of female rats. *a*, Electrolytic lesions (1.5 mA, 10 s, insulated platinum electrode 0.4 mm o.d., columns 1 and 2) or 6-hydroxydopamine lesions (2 µg base in 0.5 µl 0.9% NaCl with 0.2% ascorbic acid injected over 5 min through a cannula 0.3 mm o.d., columns 3 and 4) of the VNAB in ovariectomized rats treated with oestradiol and progesterone. Lesioned females are represented by cross-hatched columns, the receptive behaviour of lesioned females was significantly lower than controls ($P < 0.05$, Mann-Whitney *U*-test) in both cases. Coordinates for lesions were AP -0.5; L ± 0.6; V -6.5 according to König and Klippel¹⁵. *b*, VNAB lesions in intact females, showing behavioural effects during three consecutive oestrous cycles (time scale on abscissa in days). Lesioned rats showed impaired lordosis responses (receptivity) during each period of oestrus ($P < 0.05$) while proceptivity was unaffected. Each female had her vagina covered to prevent intromissions. *c*, In ovariectomized oestrogen and progesterone treated rats, procaine-induced anaesthesia of the perineum (Ana.) significantly reduced ($P < 0.05$) receptivity when compared with pre- or post-anaesthesia scores or injection of normal saline (Sal.) at the same sites in the same volume as procaine. Proceptive behaviour was unaffected.



controls (26.2 ± 2.4 days compared with 17.2 ± 1.3 days, $P < 0.05$). However, Fig. 1b shows that the behavioural consequences which followed this procedure in intact animals were very similar to those seen in ovariectomized, oestrogen-treated ones described above. Both lesioned and control animals showed proceptive behaviours every fourth day on the night preceding the presumed time of ovulation. Only the controls showed high levels of receptivity while lesioned rats responded to attempted mounts by only infrequent lordosis. Thus, ventral noradrenergic bundle lesions produced the same behavioural syndrome in animals responding to their own hormones as was observed in ovariectomized animals given oestrogen and progesterone exogenously.

One interpretation of these results is that somatosensory information received from the male during mounting was not gaining access to the neural mechanism responsible for initiating lordosis (receptive) postures in the female rat. We therefore compared these results to those from a third experiment in which we studied behaviour after anaesthetizing the skin in the female's perineal region, to which the male applies tactile stimuli during mounting. Procaine was injected at four sites (0.25 ml per site as a 2% solution) around the perivaginal area as described previously³. Figure 1c shows that this procedure produced behavioural effects remarkably similar to those following ventral noradrenergic bundle lesions, rats showing marked deficiencies in receptivity but not in proceptivity.

Although these experiments showed, in a behavioural context, that the ventral noradrenergic bundle was clearly important in the transmission of tactile information generated during sexual interaction, we wanted to know whether this system of neurones could function similarly in any other neuroendocrine context. Tactile stimulation of the uterine cervix during copulation in the rat results in daily surges of prolactin which, by extending the lifespan of the corpus luteum, induces pseudopregnancy⁷. This stimulus can be mimicked by gently stimulating the cervix with a glass rod for 40 s during proestrus⁸. In a fourth experiment we found that 8 out of 11 unlesioned females so treated (73%) became pseudopregnant judging by the occurrence of persistent basal cells in their vaginal smears which were taken daily for about a week following stimulation. However, a similar procedure applied to 9 rats with lesions of the ventral noradrenergic bundle resulted in only 2 (22%) becoming pseudopregnant, the remainder continuing to show vaginal cycles after stimulation (Fisher's exact probability test $P = 0.032$). This experiment suggests that the ventral noradrenergic pathway is concerned with somatosensory information important in neuroendocrine contexts other than those of regulating behaviour. But whether this only applies to stimuli derived from the genitalia, as explored in the present experiments, or can also extend to other parts of the body and other behavioural or physiological contexts, awaits further study.

It is known that tactile stimuli important in eliciting the receptive posture reach the brain via fibre systems running in the

anterolateral columns of the spinal cord⁹, and many of these fibres terminate in the vicinity of cell groups which contribute to the ventral noradrenergic bundle, particularly those lying in the ventro-lateral medulla and in the region medial to the superior olivary complex. It has also recently been shown that tritiated oestradiol is taken up into many noradrenergic cell bodies in the brain stem¹¹, which suggests a more direct relationship may exist between hormones which alter behaviour or pituitary secretion and this part of the noradrenergic pathway.

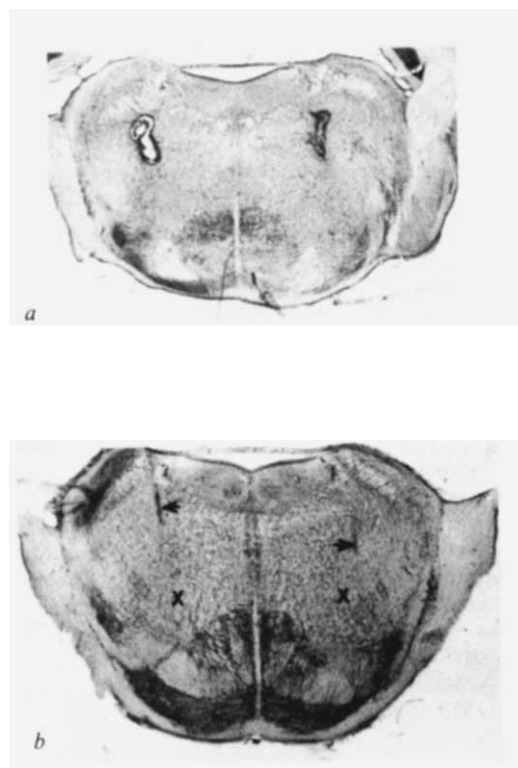


Fig. 2 Representative photomicrographs of electrolytic lesions of the VNAB (a) and the site of injection of 6-OHDA (b) which resulted in neurotoxic lesion of VNAB. In a the maximum dorsal-ventral extent of the lesion is shown, high power examination revealed necrosis and glial infiltration medial to the macroscopic lesion site. In b small glial scars can be seen (arrows) which indicate the site of descent of the micro-cannulae through which 6-OHDA was injected at depth X. No gross damage around the injection sites can be seen indicating that nonspecific effects of the injection were minimal. The neurochemical consequences of these lesions are shown in Table 1.

We suggest that ascending noradrenergic neurones carried in the ventral noradrenergic bundle and distributed to sub-cortical (hypothalamic and limbic) areas of the brain, may be an important pathway by which tactile information generated during sexual interaction modulates both behaviour and pituitary function. It becomes important to define, therefore, those sites in the brain where noradrenergic neurones exert these effects. Our preliminary evidence suggests that they may depend on the subsequent modulation of dopaminergic mechanisms: thus the switch from active to passive (proceptive to receptive) elements of the female's behaviour may be achieved by a corresponding, somatosensory-induced switch (decrease) in dopaminergic activity¹² mediated by the ventral noradrenergic pathway. Similar mechanisms may also underlie coitus (somatosensory)-induced prolactin secretion, particularly since this hormone (as well as lordosis) is under inhibitory dopaminergic control^{13,14}. Furthermore, it is possible that these sub-cortically projecting noradrenaline-containing neurones may be involved in the

Table 1 Effects of electrolytic or neurotoxin lesion on the *in vitro* uptake of ³H-noradrenaline by hypothalamic synaptosomes and slices or cerebral cortex slices

Lesion	% Decrease in active uptake of ³ H-noradrenaline <i>in vitro</i>	
	Hypothalamus	Cerebral cortex
Electrolytic	59.3%*	8% (NS)
6-Hydroxydopamine	65.8%*	13% (NS)

Incubations were performed in Krebs-Ringer bicarbonate buffer¹⁶ with ³H-noradrenaline at a concentration of 10^{-7} M. Data are expressed as % reductions compared with controls (sham lesions resulted in no significant depletions (<5%). The degree of reduction in active uptake is closely related to the degree of neuronal destruction¹⁷. NS, Not significant.

* $P < 0.01$, comparison of original counts by *t*-test.

transmission of somatosensory information in contexts wider than those reported here.

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Spontaneous and mechanically evoked activity due to central demyelinating lesion

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Patients with multiple sclerosis commonly complain of tingling and other sensory disturbances which may occur spontaneously or in response to movement. These symptoms are associated with plaques of demyelination in the corresponding sensory pathways but the physiological abnormalities underlying the symptoms are not understood. We have examined sensory fibres traversing a central demyelinated lesion resembling that of multiple sclerosis for evidence of activity which could account for the symptoms. We present here evidence for the existence of spontaneously and mechanically induced trains of action potentials propagating in both directions from the lesion.

Lesions were induced under anaesthesia (Saffan, Glaxo) in nine cats by the direct microinjection of lysophosphatidylcholine (LPC) into the dorsal column between L₁ and L₃ (ref. 1). The cats were reanaesthetized 7 to 14 days later, given atropine (300 µg) and rigidly held in a stereotactic frame. A laminectomy was performed from T₉ to L₇. Skin flaps were raised to form a recording paraffin pool, maintained at 37 ± 0.5 °C. Dorsal rootlets caudal and rostral to the lesion were isolated and raised on recording electrodes connected via unity gain cathode followers to differential preamplifiers. The data were processed using a small laboratory computer (Biomac 1000; Data Laboratories). The lesions were examined histologically.

Many of the rootlets caudal to the lesion in each of the cats contained spontaneously active units firing with fairly steady frequencies of between 15 and 45 impulses per second (Fig. 1a). Some spontaneously active units fired in bursts with, for exam-

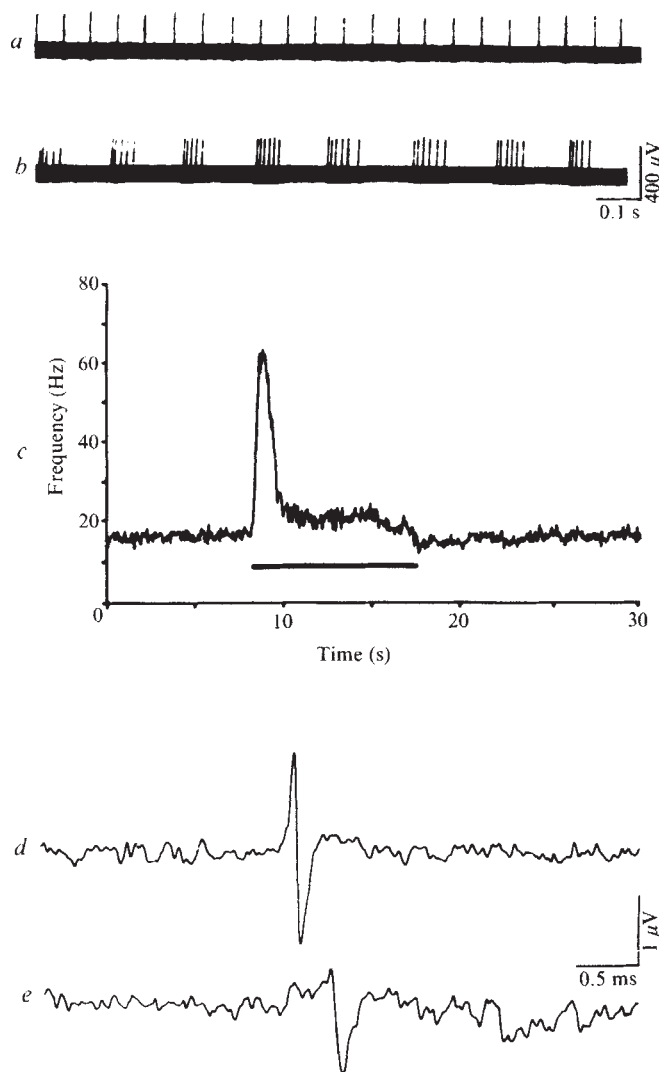


Fig. 1 *a* and *b*, Differential recordings of a steady (*a*) and a bursting (*b*) spontaneously active unit isolated in a teased dorsal root filament, caudal to the lesion, 14 days after LPC injection. *c*, Shows the firing rate of a steadily discharging, mechanosensitive unit recorded from a caudal dorsal root, 8 days after the injection of LPC. The bar marks the duration of a 0.75 mm compression of the lesion with a glass rod. *d* and *e*, Averaged records ($n = 4,096$) of the same spontaneously active unit, 14 days after LPC injection. Recordings were made 16 mm (*d*) and 6 mm (*e*) rostral to the lesion (conduction velocity = 30 m s⁻¹). The averaged was triggered from the antidromic spikes of the unit in a teased dorsal root filament caudal to the lesion. The recordings were made with an imposed delay of 2 ms.

ple groups of about 5 impulses at 110 Hz separated by intervals of approximately 0.1 s (Fig. 1b). This pattern of firing bears a striking resemblance to that of the motor discharges associated with myokymia in multiple sclerosis².

Small deformations (<1 mm) of the dorsal columns with a glass rod at the site of the lesion increased the firing rate of the spontaneously active units (Fig. 1c) and frequently recruited firing in units which were previously silent. The firing evoked by sustained deformations typically consisted of a phasic burst of activity followed by a slower, tonic discharge which subsided over about 30 s. Since the resting discharge pattern was not altered by comparable deformations of the dorsal column remote from the lesion, we conclude that the impulses were generated at the lesion in fibres which were unusually mechanosensitive. Tingling sensations, however, are likely to

depend on impulses ascending the dorsal columns and it was therefore necessary to establish the existence of abnormal centripetal as well as centrifugal activity. The difficulty of distinguishing abnormal from normal ascending activity in the dorsal columns was overcome by the use of spike-triggered averaging³ ($n = 128-16,284$) and the demonstration that, in some fibres, the descending impulses were time-locked to ascending impulses (see Fig. 1*d, e*). The averager was triggered from activity in a single nerve fibre isolated in the central end of a severed dorsal root caudal to the lesion. The input to the averager was connected to an uninsulated platinum wire which was inserted into the dorsal column rostral to the lesion using a micro-manipulator. A delay line (Mullard TDA 1022) was incorporated in the recording circuit when required. Of the 15 fibres examined with this recording arrangement we were able to find three in which there was clear evidence of synchronized trains of action potentials above and below the lesion. Two of the three fibres had quite steady firing frequencies for many hours and the other fibres fired in bursts. The relative latencies of the ascending and descending spontaneous action potentials established that they arose in the lesion. The fibres were also unusually mechanically sensitive at this site. In one fibre it was possible to determine the conduction velocity by recording the ascending action potential at two locations rostral to the lesion (Fig. 1*d, e*). From this measurement it was possible to determine the approximate site of origin of the impulses and this fell within the lesion.

The mechanism of origin of the time-locked ascending and descending impulses is uncertain. The simplest explanation is that they arose within the lesion in fibres with intact axons but abnormal myelin sheaths. Ectopic activity is known to occur in congenitally dysmyelinated axons⁴. It is, however, possible that the ascending impulses arose in surviving fibres by ephaptic excitation from spontaneous activity in adjacent severed fibres. Severed fibres exist in the LPC lesion at 14 d¹, as they do in the lesion of multiple sclerosis⁵ and are known to generate spontaneous activity whether cut centrally (our unpublished observations) or peripherally⁶. Ephaptic cross-activation has been reported in congenitally dysmyelinated spinal roots of dystrophic mice⁴. Whether demyelinated, but previously normal nerve fibres can be similarly excited remains to be determined.

The present experiments provide clear evidence of spontaneous and mechanically induced trains of action potentials arising in a central demyelinating lesion and propagating in both directions from the lesion. We think it likely that such abnormal activity contributes to some of the sensory symptoms of human disease associated with comparable lesions. For example, many patients with multiple sclerosis or compressive lesions of the cervical spinal cord experience transient paraesthesiae on neck flexion. During this manoeuvre the cervical spine changes in length by several centimetres⁷ and we have shown that much smaller deformations (<1 mm) can initiate transient abnormal discharges of a duration similar to that of the paraesthesiae. Length changes also occur in the optic nerve during eye movements and in patients with demyelinating optic neuritis such movements are sometimes associated with the perception of flashes of light⁸. The long-sustained, resting spontaneous discharges similarly provide a sufficient explanation for the continuous paraesthesiae often experienced during acute relapses of multiple sclerosis.

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Intrathecal morphine inhibits substance P release from mammalian spinal cord *in vivo*

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The central terminals of small diameter primary sensory neurones associated with the transmission of noxious cutaneous stimuli are located predominantly in the superficial dorsal horn of the spinal cord¹⁻³. Some of these neurones synthesize substance P (ref. 4) and transport this peptide to their central and peripheral terminals⁵⁻⁷. Substance P is released from the dorsal horn *in vitro* following potassium depolarization^{8,9}; from spinal cord after electrical stimulation of dorsal roots¹⁰ and from dissociated sensory neurones grown in culture¹¹. The iontophoretic application of substance P produces a long-lasting excitation of dorsal horn neurones that are also excited by noxious cutaneous stimuli¹²⁻¹⁴. One population of opiate receptors in the dorsal horn seems to be located on primary afferent terminals^{15,16}; the release of substance P from primary sensory neurones is inhibited by opiates *in vitro*^{8,11}. At present, however, there is no direct evidence that substance P is released from sensory neurones, *in vivo*, following activation of nociceptive afferents. We report here that substance P-like immunoreactivity is released from the mammalian spinal cord, *in vivo*, following chemical stimulation of sensory neurones with capsaicin and by the activation of high threshold peripheral afferents. Furthermore, release of substance P evoked by high intensity stimuli is completely inhibited by intrathecal morphine.

Experiments were performed on rats and cats, anaesthetized with chloralose-urethane and placed in a stereotaxic frame. A length of PE-10 polyethylene tubing, serving as an inflow cannula, was inserted into the subarachnoid space, via the cisterna magna, to the caudal region of the lumbar enlargement¹⁷. The spinal cord was superfused at 100 $\mu\text{l min}^{-1}$ with artificial cerebrospinal fluid (CSF) containing bovine serum albumin (120 $\mu\text{g ml}^{-1}$) and bacitracin (30 $\mu\text{g ml}^{-1}$ in rats and 300 $\mu\text{g ml}^{-1}$ in cats). In rats, superfusate was withdrawn with a push-pull syringe pump through a second cannula, placed in the cisterna magna. Superfusate from cat spinal cord was removed with a PE-90 cannula, inserted concentrically over the PE-10 cannula to the rostral region of the lumbar enlargement. Samples were collected at 10-min intervals in rats and 30-min intervals in cats.

After superfusion of the rat spinal cord in the absence of any evoking stimulus, the levels of substance P released were too low for reliable measurement in most experiments (<5 fmol per 10 min). Superfusion of the spinal cord with potassium chloride (40 mM) resulted in the release of 78 ± 9 fmol (mean $\pm$ s.e.m.; $n = 4$) substance P during the 10-min collection period. In the fraction following potassium stimulation, release of substance P returned to basal levels. Addition of 2.0 mM cobalt to the superfusing medium prevented the evoked release of substance P.

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In the cat it was possible to detect a significant release of substance P from the spinal cord in the absence of any evoking stimulus: 7–15 fmol were measured in each 30-min collection period with very little variation during the course of an experiment (Fig. 1). To examine whether substance P is released from the spinal cord following activation of specific afferent fibre populations, we stimulated the cat sciatic nerve bilaterally, while superfusing segments of the spinal cord receiving sensory input from the sciatic nerve. During stimulation, the compound action potential was monitored to determine the stimulus intensity required to activate A α β fibres alone, and that required to recruit A δ and C fibres. At stimulus intensities sufficient to recruit only low threshold A α β afferents, there was no measurable increase in the release of substance P (Fig. 1). The collaterals of large myelinated fibres that are activated by low intensity stimuli terminate with deeper laminae of the dorsal horn. Thus, by superfusing the surface of the spinal cord, we cannot exclude that substance P is actually released from those terminals, but cannot diffuse into the spinal superfusate. Increasing the stimulus intensity to activate A δ and C fibres

produced a 4.9 ± 0.6 fold (mean $\pm$ s.e.m.; $n = 4$) increase in substance P release (Fig. 1). In the fraction following stimulation, the release of substance P returned to pre-stimulation values.

We have also examined whether the spinal application of morphine at concentrations known to elicit analgesia¹⁸ can affect substance P release from cat spinal cord *in vivo*. Following superfusion with 5×10^{-4} M morphine sulphate (equivalent to 427.5 μ g morphine base superfused over a 30-min period), the resting release of substance P was substantially decreased, in two animals to levels below the sensitivity of the assay (Fig. 1). Furthermore, bilateral stimulation of the sciatic nerve at intensities that clearly caused the release of substance P before the addition of morphine now failed to increase the release of substance P (Fig. 1). Following intraperitoneal injection of naloxone hydrochloride (1 mg per kg), stimulation of the sciatic nerve at the same intensity in the continued presence of intrathecal morphine, fully restored the evoked release of substance P (Fig. 1).

Peripheral application of the homovanillic acid derivative, capsaicin, produces a severe burning pain¹⁹ that reflects the activation of C fibres in sensory nerves²⁰. Subcutaneous administration of capsaicin to neonatal rats results in the degeneration of small sensory neurones²¹, some of which contain substance P²².

Capsaicin also depletes substance P from the dorsal horn of adult rats^{23,24}. Administration of capsaicin directly onto the spinal cord, *in vitro*, causes a calcium-dependent release of substance P^{9,25}. In the present experiments, superfusion of the cat spinal cord with 3×10^{-4} M capsaicin produced a greater than tenfold increase in the release of substance P (5 experiments, Fig. 1). The actions of capsaicin in the spinal cord seem to be restricted to nociceptive primary sensory neurones^{23,26}. Thus, the demonstration of a capsaicin-evoked release of substance P from cat spinal cord, *in vivo*, provides further evidence that substance P may be involved in the transmission of noxious peripheral stimuli.

Our results with sciatic nerve stimulation provide direct evidence that substance P is released from mammalian spinal cord *in vivo* by activating nociceptive sensory afferents. The naloxone-reversible inhibition of substance P release by morphine applied directly to the spinal cord at concentrations known to elicit analgesia, may represent one substrate for the direct spinal analgesic actions of the opiates¹⁸. The opiate inhibition of substance P release reported by Mudge *et al.*¹¹ is a direct effect on sensory neurones. These authors also found that enkephalin could inhibit calcium influx into cultured sensory neurones by modulating voltage-sensitive channels and suggested that inhibition of substance P release from terminals could be mediated by the same mechanism.

The precise relationship between enkephalin and substance P-containing neuronal elements in the dorsal horn is still unclear. Substance P is highly concentrated within lamina I of the dorsal horn, where primary afferent terminals are rarely postsynaptic in axo-axonal contacts³. Furthermore, although there are a variety of enkephalin-containing elements within the superficial dorsal horn^{27–29} few of these seem to contribute to axo-axonal contacts with primary afferents³⁰. It remains to be seen if other neurotransmitters present in the dorsal horn that can elicit direct spinal analgesia^{31,32} also inhibit the release of substance P *in vivo*.

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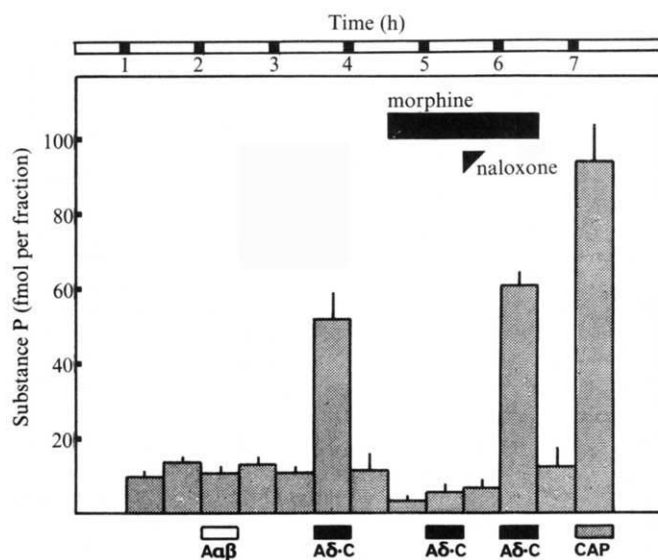


Fig. 1 Release of substance P from superfused cat spinal cord in response to sciatic nerve stimulation and capsaicin. Cats were anaesthetized with chloralose-urethane (100 mg per kg) and prepared with a tracheal tube and jugular and carotid catheters. An incision was made in the cisterna magna and the perfusion cannula was inserted after retraction of the arachnoid layer. The perfusion cannula consisted of a length of polyethylene PE-90 (1.0 mm outer diameter) tubing through which was passed a length of polyethylene PE-10 tubing. The PE-10 served as inflow cannula while the PE-90 was used as collection cannula. Both cannulae were inserted through the retracted incision in the cisterna membrane 37–40 cm down the spinal cord (upper sacral region) and the PE-90 cannula was then retracted 10 cm. Perfusion was therefore localized to the upper sacral and lumbar spinal cord. The position of the inflow and outflow cannulae was determined by X rays after infusion of radio-opaque dye. The sciatic nerve was exposed and prepared for stimulation and recording of the compound action potential. Stimulation of the nerve was performed with rectangular pulses (3–4 V, 0.05 ms, 50 Hz for activation of A α and A β fibres and 40–50 V, 0.05 ms, 50 Hz for recruitment of A δ and C fibres). Superfusate in all experiments consisted of NaCl, 151.1 mM; KCl, 2.6 mM; MgSO₄, 0.9 mM; CaCl₂, 1.3 mM; NaHCO₃, 21.0 mM; K₂HPO₄, 2.5 mM, gassed with 95% O₂ and 5% CO₂ before perfusion. Perfusion samples (3 ml) were collected into glacial acetic acid (final concentration of 2 N) and immediately frozen and lyophilized. Samples were reconstituted in 1.0 ml assay buffer, neutralized and aliquots of each fraction were used to determine the content of substance P, by radioimmunoassay, using antibody R6P with a sensitivity of 1.5 fmol per assay tube³³. Serial dilutions of synthetic substance P and immunoreactivity in superfusate samples produced parallel dilution curves. Each value is the mean $\pm$ s.e.m. from four separate experiments.

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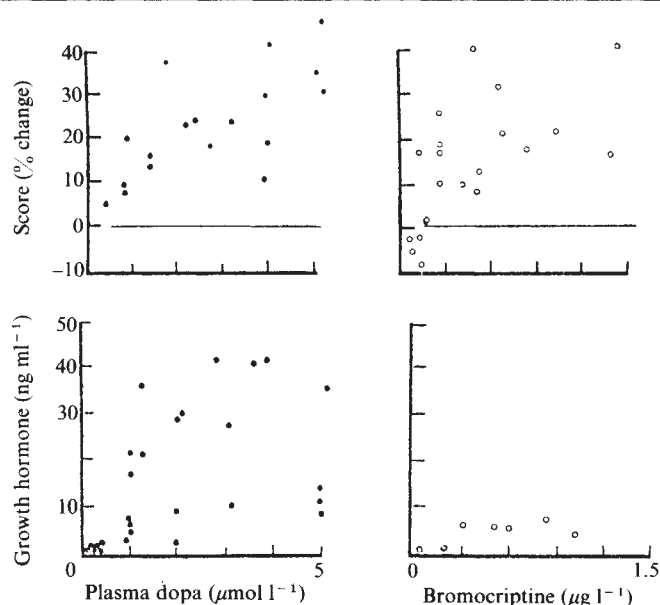


Fig. 1 Peak plasma dopa ($\mu\text{mol l}^{-1}$) or bromocriptine ($\mu\text{g l}^{-1}$) level, peak percentage reduction in parkinsonian disability score (score units), and peak elevation of plasma growth hormone level (ng ml^{-1}) after single oral doses of levodopa (1 g) or bromocriptine (12.5–100 mg) in subjects with parkinsonism. With dopa, relationship between plasma drug level, motor and growth hormone effect; $r = 0.69$, $P < 0.01$, $n = 18$ and $r = 0.50$, $P < 0.05$, $n = 27$, respectively; and with bromocriptine, $r = 0.50$, $P < 0.05$, $n = 20$ and $r = 0.10$, not significant, $n = 7$.

The role of D-1 and D-2 receptors

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Dopamine receptors in intracerebral motor and endocrine systems^{1,2} have been divided into two main types, D-1 and D-2, dependent on the presence or absence of adenylate cyclase linkage³. Here we have investigated a number of dopamine agonist and antagonist drugs in man that have different actions on D-1 and D-2 receptors in animals. Motor and endocrine effects in parkinsonian subjects seem to depend on drug interaction with D-2, but not D-1, receptors. These results may have important implications for the design of anti-parkinsonian and antipsychotic agents.

Many dopamine agonists and antagonists have selective actions on D-1 and D-2 systems, as determined by adenylate cyclase stimulation^{4–6} and membrane binding studies^{7–9}, but examination of possible differences in motor, hormonal and other behavioural effects of these compounds in man has proved difficult because of individual variations in drug pharmacokinetics and the response¹⁰. Despite these problems, we have attempted to establish the magnitude of the motor, growth hormone and prolactin responses to known plasma levels of levodopa and the dopamine-like ergot derivatives, bromocriptine and lisuride^{11,12}. Dopamine in low concentration is an agonist at D-2, and in higher concentration at D-1 receptor sites¹³, but the ergot derivatives which act as agonists at D-2 receptors in micromolar concentrations have antagonist actions in nanomolar concentrations on striatal D-1 receptors^{14,15}. We have also studied the effects of pimozide, a non-selective dopamine receptor antagonist¹⁶, *cis*-flupenthixol, an effective antipsychotic with D-1 antagonist effects on the striatum^{8,17}, (blockade of adenylyl cyclase activity shows a linear relationship with ³H-*cis*-flupenthixol binding in striatal tissue), and the D-2 antagonists metoclopramide, sulpiride, oxiperomide and tiapride¹⁸, on levodopa-stimulated motor and hormonal changes. These drugs were given to subjects with Parkinson's

disease, in which dopamine sensitive adenylate cyclase activity is reduced by at least 50% (ref. 19), although the number of D-2 receptor sites in the striatum is increased, as determined by ³H-haloperidol binding²⁰. Following levodopa treatment, specific ³H-spiperone binding approaches normal values²¹. Receptor changes and the loss of presynaptic neurones must influence the motor response to levodopa in parkinsonism: for instance, normal subjects do not readily develop levodopa dyskinesias²². Pituitary and hypothalamic dopamine receptor assays have not been reported in parkinsonian patients, but prolactin and growth hormone responses to levodopa are similar to those in age- and sex-matched normal controls²³.

Levodopa, bromocriptine and lisuride caused dose-dependent improvement in tremor, akinesia and rigidity, together with dyskinesias, a rise in growth hormone level and a fall in prolactin level in most subjects. Emesis, visual and auditory hallucinosis and delusions occurred with each drug. Bromocriptine and lisuride, but not levodopa, occasionally induced sleep. Systolic and diastolic erect and supine blood pressure fell by 10–20 mm Hg following levodopa, 1 g orally (p.o.), bromocriptine, 100 mg p.o., and lisuride, 0.01 mg intravenously (i.v.). Neither the

Table 1 Timing and duration of response to dopamine agonists

	Peak drug plasma level (min)	Peak therapeutic effect (min)	Peak plasma growth hormone elevation (min)	Peak plasma prolactin depression (min)
Levodopa, 1g p.o.	80	90 (180)	90 (120)	45 (150)
Bromocriptine, 100 mg p.o.	100	130 (480)	170 (120)	60 (480)
Lisuride, 0.01 mg i.v.	—	30 (120)	45 (120)	60 (240)

Values are means after single doses of levodopa, bromocriptine and lisuride in a subject with Parkinson's disease. The total duration of each response is shown in parentheses.

Table 2 Effect of levodopa, bromocriptine and lisuride in Parkinson's disease, and of antipsychotic drugs on levodopa-mediated endocrine changes

	No. of patients	Mean peak plasma levodopa level ($\mu\text{mol l}^{-1}$)	Parkinsonism reduction in disability score	Dyskinesia mean dyskinesia score	Growth hormone rise in plasma level (ng ml^{-1})	Prolactin fall in plasma level (%)
Levodopa, 1 g p.o.	(18)	4.5 ± 1	31 ± 4	4 ± 2	20 ± 3	41 ± 10
Bromocriptine 100 mg p.o.	(4)	—	37 ± 8	7 ± 2	$6 \pm 4^*$	41 ± 11
Lisuride, 0.15 mg i.v.	(4)	—	25 ± 5	1 ± 1	22 ± 8	43 ± 11
Levodopa, 1 g and pretreatment with antagonists at stated intervals:						
Metoclopramide, 60 mg p.o. 1 h	(14)	5.1 ± 1	21 ± 5	2 ± 1	$6 \pm 1^\dagger$	48 ± 15
Pimozide, 6 mg p.o., 1 h	(11)	4.4 ± 1	$13 \pm 4^\dagger$	1 ± 1	15 ± 5	
Tiapride, 200 mg p.o., 1 h	(2)	5.2 ± 1	26 ± 4	1 ± 1	16 ± 8	55
Oxiperomide, 10 mg p.o. 1 h	(5)	4.9 ± 2	27 ± 5	1 ± 1	24 ± 5	46 ± 12
cis-flupenthixol, 3 mg p.o. 4 h	(5)	—	17 ± 3	0	20 ± 3	76 ± 6
Sulpiride, 400 mg p.o. 2 h	(5)	—	$14 \pm 2^\ddagger$	0	$2 \pm 1^\ddagger$	50 ± 7
Placebo (sulpiride), p.o. 2 h	(5)	—	31 ± 4	2	18 ± 3	62 ± 12

Mean ($\pm$ s.e.m.) peak motor and hormonal changes after single doses of dopamine agonists, and after single doses of levodopa, 1 g p.o. preceded at the stated intervals by a range of antipsychotic drugs are shown. Motor responses: peak reduction in parkinsonian disability score and peak dyskinesia score (both King's College Hospital rating scales) are shown. Hormonal responses: peak increase in plasma growth hormone level (ng per ml) and peak reduction in plasma prolactin level (maximum percentage fall from basal level in case of agonists, and from peak stimulated level in case of antagonists) are shown. All observations are over a 4-h period.

* Rise after bromocriptine less than after levodopa or lisuride ($P < 0.05$ in each case, Student's *t*-test).

† Reduction in anti-parkinsonian effect after pimozide, and in growth hormone peak after metoclopramide, compared with levodopa alone ($P < 0.01$ in each case, Student's *t*-test).

‡ Reduction in anti-parkinsonian effect and growth hormone peak after sulpiride compared with levodopa and placebo ($P < 0.025$, Wilcoxon's signed rank test).

motor nor the hormonal responses were biphasic, that is, deterioration in parkinsonism did not precede improvement, and elevation of prolactin level or depression of growth hormone level did not precede the opposite effect. Peak motor and hormonal responses were simultaneous with peak plasma levels of each drug, although each compound had a different pharmacokinetic profile (Table 1). In equipotent anti-parkinsonian doses, the peak rise in plasma growth hormone level with levodopa ($20 \pm 3 \text{ ng ml}^{-1}$) and lisuride ($22 \pm 6 \text{ ng ml}^{-1}$) was greater than with bromocriptine ($6 \pm 3 \text{ ng ml}^{-1}$); $P < 0.05$ in each case.

The dopamine antagonist drugs had comparable motor and hormonal effects (Table 2). Sulpiride (400 mg) and pimozide (6 mg) were most active, and tiapride (200 mg) and oxiperomide (10 mg) were least active in producing antagonism of levodopa-induced motor and hormonal responses. All antagonists caused hyperprolactinaemia, and prolactin levels subsequently fell following levodopa (1 g). All antagonists reduced the severity of levodopa-induced dyskinesias, but also reduced the therapeutic effect of levodopa, with the exception of tiapride (200 mg) and oxiperomide (10 mg). No antagonist prevented the hypotensive action of levodopa.

The motor, endocrine and behavioural effects of levodopa, bromocriptine and lisuride in Parkinson's disease are thus very similar despite the different effects of these drugs on D-1 and D-2 receptors in animal models. The low peak plasma growth hormone response to bromocriptine compared to equipotent anti-parkinsonian doses of levodopa or lisuride may be due to the action of bromocriptine as a weak noradrenergic antagonist²³. However, lisuride has similar adrenergic effects to bromocriptine¹².

In animal tissues, bromocriptine has dose-dependent antagonist or agonist effects on D-1 receptors, and in behavioural studies causes reduced exploratory activity before motor activation²⁴. These changes have been attributed to separate pre- and post synaptic effects of bromocriptine²⁵, but a dual response with motor and hormonal inhibition at low plasma levels, and stimulation at high levels, is not evident in Parkinson's disease. However, bromocriptine may act as a partial rather than a complete agonist in the motor system, as clinical improvement with very high plasma bromocriptine levels is not always as great as that accompanying lower levels¹⁰.

As with dopamine agonists, there are no distinctions between the actions of selective D-2 and non-selective D-1 and D-2 receptor antagonists in parkinsonism, apart from differences in potency. Metoclopramide and pimozide are both competitive antagonists to the anti-parkinsonian and growth hormone-elevating actions of levodopa, despite different effects on striatal D-1 and D-2 systems, and the other D-2 antagonists also block these responses. Oxiperomide and tiapride prevent levodopa-induced stereotypies but not motor hyperactivity in rodents²⁶, and in critical dosage have specific antidyskinetic effect in parkinsonism without impairing the therapeutic action of levodopa²⁷, although the apparent selectivity of these drugs has not been satisfactorily explained.

The motor and endocrine effects of levodopa, bromocriptine, lisuride and also of different dopamine antagonists in parkinsonism can be attributed to the effects of these drugs on D-2 non-adenylate cyclase linked receptors. The physiological significance of the dopamine-sensitive adenylyl cyclase is obscure. Phosphodiesterase inhibitors—including caffeine and oxpentifylline—given to retard the breakdown of cyclic AMP have little or no therapeutic effect in parkinsonism²⁸. Hormonal responses to dopamine in peripheral tissues, as shown for example by the release of parathormone from the parathyroid, are mediated by dopamine-stimulated increase in intracellular cyclic AMP²⁹ and cyclic AMP can mimic the hyperpolarizing effect of dopamine when applied directly to ganglionic neurones³⁰. However, prolactin suppression by dopamine in pituitary cells does not involve adenylyl cyclase mechanisms¹⁵. The presence of this enzyme in striatal neurones may be more important in determining the intracellular rather than the electrophysiologically significant membrane response to dopamine.

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α -Adrenergic receptors modulate β -receptor affinity in rat kidney membranes

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Adrenergic receptors were first classified into two classes— α and β —on the basis of the relative pharmacological potencies of agonist compounds¹, and this classification has been supported by subsequent studies. In some tissues, such as the heart and liver^{2,3}, they exert similar physiological responses, and in other tissues, such as the uterus and vasculature, they have opposing roles^{4,5}. The occurrence of both classes of receptor in the same tissue leads to the problem of what determines whether the overall response observed is α -type or β -type, as adrenaline and noradrenaline bind to both receptor classes. Furthermore, direct binding studies have demonstrated that the two receptor classes are distinct and separate entities⁶. We show here that stimulation of α -receptors in renal membranes causes a specific decrease in the affinity of the agonist compound isoprenaline for β -receptors in the same membranes. This demonstrates that interactions occur between renal α - and β -adrenergic receptors. Such interactions may modulate the response of the kidney to sympathetic stimulation.

Adult Sprague-Dawley rats were killed by cervical dislocation. Kidneys were removed immediately and homogenized in 0.25 M sucrose in buffer (50 mM Tris-HCl, pH 8.1, 10 mM MgSO₄). The homogenate was centrifuged twice at 2,000g for 5 min and the final supernatant was centrifuged at 30,000g for 15 min. The pellet was washed three times with buffer and finally resuspended in buffer at a protein concentration of 5–10 mg ml⁻¹. Binding studies were carried out as described in detail elsewhere⁷. Renal membranes (0.1 ml) were incubated with 5 nM ³H-dihydroalprenolol (³H-DHA, Searle Nucleonics) in a final volume of 0.5 ml in 50 mM Tris-HCl, pH 8.1, 10 mM MgSO₄, 10⁻⁴ M pyrocatechol and 0.1% ascorbic acid. Incubation was carried out at 37°C for 10 min and was terminated by filtration through Whatman GF/C glass fibre filters. The filters were washed with 10 ml ice-cold buffer. Specific binding was defined as binding displaceable by 10⁻⁵ M isoprenaline and constituted 60–70% of the total binding at

5 nM ³H-DHA. The characteristics of ³H-DHA binding sites in these conditions were those expected of β -receptor binding and have been described in detail elsewhere⁷. The relative potencies of adrenergic agonists in competing for binding were isoprenaline > (–)adrenaline $\geq$ noradrenaline > (+)adrenaline = dopamine. (–)Propranolol was 100 times more potent than (+)propranolol. The α -adrenergic agents phenylephrine and phentolamine competed for binding only at concentrations greater than 10⁻⁵ M. No competition for ³H-DHA binding by clonidine was observed at concentrations up to 10⁻⁴ M. Renal membranes contained 33 $\pm$ 3 (s.e.m.) fmol per mg of β -receptors, measured by ³H-DHA binding. The affinity of the receptors for ³H-DHA was 1.5 $\pm$ 0.4 nM (n = 6). Receptor concentration and affinity were calculated by Scatchard analysis⁸.

The affinity of the β -adrenergic agonist isoprenaline for renal β -receptors measured in competition experiments with ³H-DHA was decreased in the presence of 10⁻⁶ M clonidine from 72 $\pm$ 4 nM to 185 $\pm$ 20 nM (average of 10 experiments; P < 0.005) (Fig. 1). This effect of clonidine was specific for agonists as clonidine had no effect on the affinity of the antagonist propranolol for the same membrane receptors (Fig. 1). Similarly, clonidine had no effect on ³H-DHA binding in the absence of isoprenaline. ³H-DHA bound in the presence of 10⁻⁶ M clonidine was 96% $\pm$ 4.4 of controls containing no clonidine. The addition of clonidine also caused a change in the Hill coefficient calculated from the isoprenaline displacement curves. In the presence of 10⁻⁶ M clonidine, the Hill coefficient increased from 0.75 $\pm$ 0.05 to 1.04 $\pm$ 0.05 (average of 10 experiments; P < 0.005). A Hill coefficient of less than one indicates either multiple classes of binding sites or negative cooperativity. K_i values measured in such conditions represent the average isoprenaline affinity at 50% of maximum isoprenaline binding.

The reduction of β -adrenergic agonist affinity was found to be a specific α -adrenergic receptor-mediated effect. The effect of clonidine was concentration dependent and was maximal at 10⁻⁸–10⁻⁷ M. A decreased isoprenaline affinity was observed with clonidine concentrations as low as 10⁻⁹ M (Table 1). Moreover, the effect was due to the α -adrenergic agonist action of clonidine, as a similar reduction in isoprenaline affinity occurred with another α -agonist compound, phenylephrine. The α -adrenergic antagonist phentolamine antagonized the effect of clonidine (Table 1). Phentolamine alone had no effect on isoprenaline affinity.

In many different tissue preparations, the affinities of β -receptors for agonist compounds are decreased in the presence

Table 1 Modulation of isoprenaline affinity for renal β -adrenergic receptors

Additions	K_i isoprenaline (nM)	No. of experiments
None	72 $\pm$ 4	10
10 ⁻⁶ M Clonidine	185 $\pm$ 20*	10
10 ⁻⁷ M Clonidine	176 $\pm$ 31†	4
10 ⁻⁸ M Clonidine	172 $\pm$ 11‡	4
10 ⁻⁹ M Clonidine	112 $\pm$ 15†	4
10 ⁻⁵ M Phenylephrine	210 $\pm$ 42‡	4
10 ⁻⁶ M Clonidine plus 10 ⁻⁵ M phentolamine	105 $\pm$ 8§	4
10 ⁻⁴ M GTP	245 $\pm$ 12‡	4
10 ⁻⁴ M GTP plus 10 ⁻⁶ M clonidine	215 $\pm$ 31§	4

Values are mean $\pm$ s.e.m. P values were calculated using a paired t -test. K_i values were calculated using the formula: $K_i = I_{50}(1 + L/K_L)$, where I_{50} is the concentration of the drug causing 50% inhibition of binding. L and K_L are, respectively, the concentration and the dissociation constant of the ³H-DHA.

* P < 0.005 relative to controls; † P < 0.025 relative to controls; ‡ P < 0.01 relative to controls; § P < 0.05 relative to 10⁻⁶ M clonidine; § NS relative to 10⁻⁴ M GTP.

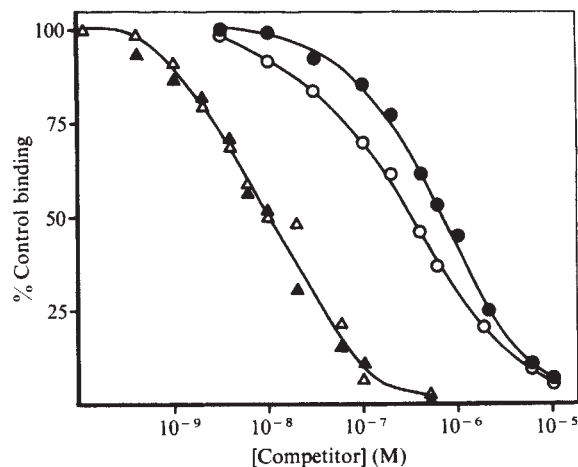


Fig. 1 Effect of clonidine on the affinities of isoprenaline and propranolol for rat kidney β -receptors. ^3H -Dihydroalprenolol (5 nM) was incubated with renal membranes in the presence of increasing concentrations of isoprenaline or propranolol and the percentage of control specific binding was determined. A typical experiment is shown. Average values of isoprenaline affinity (K_T) in the presence and absence of clonidine in 10 experiments are given in Table 1. $\circ$, Isoprenaline; $\bullet$, isoprenaline and 10^{-6}M clonidine; $\triangle$, propranolol; $\blacktriangle$, propranolol and 10^{-6}M clonidine.

of GTP and certain GTP analogues⁹. In rat kidney membranes isoprenaline affinity was reduced by 10^{-4}M GTP. The effects of clonidine and GTP were not additive, for in the presence of 10^{-4}M GTP, clonidine (10^{-6}M) did not further reduce isoprenaline affinity (Table 1).

There are marked differences in the characteristics of agonist and antagonist binding^{10,11}. Receptor affinity for antagonists remains remarkably constant and the Hill coefficient for antagonist binding is generally close to 1.0, indicating a single class of non-interacting binding sites. The Hill coefficient for agonist binding is generally less than 1.0. Also, agonist affinity can be modulated by a variety of agents including guanyl nucleotides⁹, Mg^{2+} ion¹² and cholinergic agonists¹³. In general, these factors are thought to influence the apparent binding affinity of agonists through a primary effect on adenylate cyclase or the coupling mechanism between receptor and cyclase¹⁴; renal α -adrenergic receptors inhibit adenylate cyclase¹⁵. It is likely that the observed reduction of β -adrenergic agonist affinity in renal membranes by α -adrenergic agonists is also related to an effect on adenylate cyclase.

The kidney contains a functionally heterogeneous population of β -receptors. Although there is little direct evidence, both α - and β -receptors are thought to be present in the renal vasculature¹⁶ on the juxtaglomerular cells^{17,18} and β -receptors have been demonstrated on renal tubules¹⁹. The observed interaction between α - and β -receptors in renal membranes probably occurs in only one of these functional groups. This may explain why the observed shift in isoprenaline affinity is small compared with that caused by GTP in other tissues^{9,12}. However, the lack of any additive effect of clonidine and GTP suggests that these two agents influence the same receptor population. This interaction between α - and β -receptors seems to be specific to the kidney, as we have been unable to demonstrate any effect of clonidine on isoprenaline affinity in cardiac membranes.

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Cleavage of endorphins to des-Tyr endorphins by homogeneous bovine brain aminopeptidase

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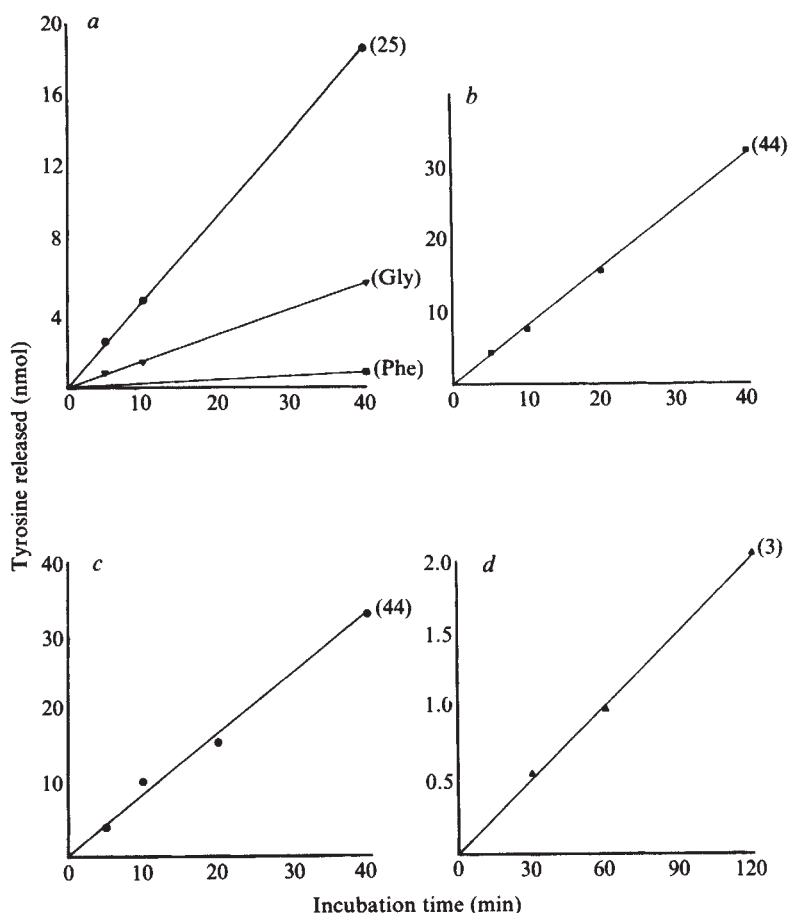
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Attention has recently focused on the possible biological roles of peptides related to β -lipotropin (β -LPH)^{1,2}. In particular, observations suggest that certain peptide fragments derivable from β -LPH, including γ -endorphin (β -LPH_{61–77}) and α -endorphin (β -LPH_{61–76}), may be involved in various modes of behavioural adaptation^{2,3}. Structure–activity studies on behavioural actions of γ -endorphin, an opioid peptide, revealed that the omission of N-terminal tyrosine resulted in a non-opioid peptide, des-Tyr- γ -endorphin, which exhibited greater behavioural activity than γ -endorphin³. Subsequent biochemical studies provided evidence for the association with brain synaptic plasma membranes of peptidase activities which could produce γ - and α -endorphins and their des-Tyr derivatives from exogenous β -endorphin⁴. This and earlier observations^{4–6} suggest that regulatory physiological events are focused on the N-terminal tyrosine residue; enzymatic reactions exerted at this residue are thus of great interest. We recently purified to homogeneity an aminopeptidase from bovine brain which catalyses the hydrolysis of Met⁵- or Leu⁵-enkephalin by sequential release of amino acids from the amino terminus of the enkephalin molecule⁷. The ability of this enzyme, purified from bovine⁷, monkey⁸ or rat⁹ brain, to use endorphins as substrates has not been previously examined. We now present quantitative studies which show that homogeneous bovine brain enkephalin aminopeptidase catalyses the hydrolysis of the N-terminal tyrosine from γ -, α - and β -endorphins with substantial turnover numbers and yields only the des-Tyr derivatives. These results suggest that this enzyme may be involved in regulating the concentrations of opioid- and opioid-derived biologically active peptides in the brain.

We incubated homogeneous bovine brain aminopeptidase, previously shown to catalyse the hydrolysis of Met⁵- and Leu⁵-enkephalins⁷ ($K_m = 22\text{ }\mu\text{M}$, $V_{max} = 20\text{ }\mu\text{mol per min per mg enzyme for the Tyr}^1\text{-Gly}^2\text{ bond}$), with synthetic α -, β - and γ -endorphins, and compared their hydrolysis with that of Met⁵-enkephalin in the same experimental conditions by subjecting aliquots of the incubation mixtures to amino acid analysis at various times of incubation. All the commercial synthetic peptides were tested before use by HPLC and amino acid analysis and judged to be pure. The enzymatic digestions were carried out in incubation conditions in which linear rates of

Fig. 1 Release of tyrosine from Met⁵-enkephalin and α -, β -, and γ -endorphin catalysed by bovine brain aminopeptidase. Reaction mixtures containing 50 mM Tris-HCl buffer, pH 7.4, 0.5 mM substrate (Peninsula) and bovine brain aminopeptidase in a total volume of 150 μ l were incubated at 37 °C. At the time points indicated, a 35- μ l aliquot was withdrawn, diluted to 0.5 ml with water and boiled for 1 min. The boiled solution was filtered through a 0.45- μ m Millipore filter, washed with 1.5 ml of water and the combined effluents lyophilized. The lyophilized samples were dissolved in 100 μ l of sodium formate buffer (0.2 M in sodium ion) at pH 2.21 and applied directly to an amino acid analyser microbore single column system containing DC-4A cation exchange resin (Pierce). The amino acids were eluted using a three-buffer programme: 0.2 M sodium formate pH 3.28, 0.2 M sodium formate pH 4.38, and 0.2 M sodium borate pH 10.2. The amino acids were identified and quantified by comparison with standards run at various intervals between samples. Analyses were carried out on an Aminco Aminolyzer fluorometric liquid chromatography system equipped with a Shimadzu digital integrator. The fluorogenic reagent used was a *o*-phthalaldehyde. *a*, Tyrosine and glycine release from Met⁵-enkephalin using 23 ng of enzyme; *b*, tyrosine release from α -endorphin using 115 ng of enzyme; *c*, tyrosine release from γ -endorphin using 115 ng of enzyme; *d*, tyrosine release from β -endorphin using 172 ng of enzyme. The values in parentheses represent the percentage of the substrate degraded.



hydrolysis were measured. Control incubations in which enzyme and substrate, respectively, were omitted, or in which boiled enzyme was used, revealed that no free amino acids were generated by non-enzymatic breakdown of substrate or by enzyme autolysis.

As shown in Fig. 1, homogeneous bovine brain aminopeptidase catalyses the release of the N-terminal tyrosine residue from Met⁵-enkephalin and from α -, γ - and β -endorphins. As shown in Table 1, the rate of hydrolysis of α - and γ -endorphins was approximately 30% that of Met⁵-enkephalin whereas β -endorphin was hydrolysed at a much lower rate—0.5% relative to Met⁵-enkephalin. It is interesting that whereas glycine was also released from Met⁵-enkephalin at about one-third the rate of tyrosine, no glycine was liberated from α - or γ -endorphin, even in conditions in which over 40% of the tyrosine was released. These results suggest that, unlike des-Tyr-Met⁵-enkephalin, des-Tyr- α - and des-Tyr- γ -endorphins adopt a conformation resistant to further degradation by the aminopeptidase. This resistance could contribute to the metabolic stability of these peptides and thus to their biological activity. Note also that the homogeneous bovine brain aminopeptidase exhibited substantial turnover numbers for the hydrolysis of the N-terminal tyrosine of α - and γ -endorphins, 689 and 634 min⁻¹ relative to 2,040 min⁻¹ for Met⁵-enkephalin (Table 1). This rapid rate of reaction with these endorphins raises the possibility of an *in vivo* role for the aminopeptidase in partitioning peptide fragments derived from β -endorphin between opioid and non-opioid pathways in response to functional demands. The rate of release of N-terminal tyrosine from β -endorphin was much lower than that for the other endorphins; although the extent of cleavage was relatively small in the conditions used, the enzyme seemed to produce only the des-Tyr fragment of β -endorphin. Whether this reaction, which would produce a non-opioid derivative of β -endorphin, is of physiological significance, for example, by being modulable by regulatory agents, is a subject for further study.

Our results provide new biochemical evidence in support of the hypothesis that des-Tyr α - and des-Tyr- γ -endorphins may have a role in brain function, as we have observed that a homogeneous brain aminopeptidase catalyses the cleavage of the N-terminal tyrosine residue from α - and γ -endorphins at a rapid rate to yield the des-Tyr derivatives with no further cleavage of the peptide. The relationship between this activity and the production of des-Tyr- α - and des-Tyr- γ -endorphins from β -endorphin by a rat brain "synaptic plasma membrane" (SPM) fraction observed by Burbach *et al.*⁴, is not known. However, in the latter study, marker enzyme data were not presented to aid in understanding to what extent the vesiculation known to accompany the preparation of such membranes may have resulted in the inclusion of synaptoplasmic elements, possibly including the soluble aminopeptidase which we have described. It will be interesting to compare the properties of the SPM-associated production of des-Tyr α - and des-Tyr- γ -endorphins with those tabulated for our homogeneous enzyme preparation in terms of sensitivity to mercurials, puromycin and chelators. On the other hand, it may be that these are separate

Table 1 Rate of tyrosine release from Met⁵-enkephalin and α -, β - and γ -endorphin by bovine brain aminopeptidase

Substrate	Rate of tyrosine release (μ mol per min per mg enzyme)	Turnover number (min ⁻¹)
Met ⁵ -enkephalin	20.4 (6.1)	2,040
α -Endorphin	6.5	634
γ -Endorphin	6.9	689
β -Endorphin	0.10	10

The data from Fig. 1 were used to calculate the rate of tyrosine release. The value in parentheses is the rate of glycine release.

*For hydrolysis of the Tyr¹-Gly² bond; molecular weight of the enzyme, 100,000 (ref. 7).

activities, and that the soluble aminopeptidase acts on a different metabolic pool of endorphins. *A priori*, based on our lack of knowledge of the modes of action of peptides in intercellular communication in the nervous system, there is no reason to assume that soluble enzymes metabolizing peptides could not be importantly involved in the use of peptides as primary output signals. In addition, many enzymes formerly considered to be soluble on the basis of extractability properties are now known to exhibit transient associations with subcellular organelles, such as hexokinase with mitochondria¹⁰ and choline-acetyltransferase with synaptic vesicles¹¹, and are solubilized from such organelles by metabolites as part of regulatory intracellular processes. It will be of interest to investigate brain peptidase activities from this perspective.

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Identification of a tetrodotoxin-sensitive Na⁺ channel in a variety of fibroblast lines

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The action potential Na⁺ ionophore of excitable cells can be activated either by alkaloid compounds such as veratridine, or by small polypeptide toxins extracted from scorpion venom or sea anemone^{1,2}. One of the main features of this Na⁺ channel is that it is blocked by tetrodotoxin (TTX). However, we report here that during analysis of Na⁺ influx in resting fibroblasts, we found that a variety of fibroblast lines also possess a TTX receptor. Veratridine and sea anemone toxin act synergistically to stimulate Na⁺ influx 7 to 10-fold in hamster and rat fibroblasts. As in excitable cells, this toxin-stimulated Na⁺ influx is blocked by TTX. Addition of serum to hamster fibroblasts arrested in G₀ stimulates Na⁺ influx three-fold. Observations that TTX does not prevent serum-activated Na⁺ influx, initiation of DNA synthesis and cell proliferation suggest that the fast Na⁺ channel which we have identified in fibroblasts is not involved in growth control.

The gating system of the Na⁺ channel in excitable cell systems such as nerve, skeletal muscle, cardiac cell and neuroblastoma is altered by veratridine and sea anemone toxin II^{3–5}. These toxins also reveal 'silent' channels⁶. Figure 1 shows that the well known action of these toxins on excitable membranes is also observed in fibroblasts. The kinetics of ²²Na⁺ uptake in the Chinese hamster lung fibroblast line CC139 (ATCC) are shown in Fig. 1a. Veratridine at 100 µM or sea anemone toxin at 10 µM have no effect on ²²Na⁺ influx. However, when added together, these toxins effect a 5–10-fold stimulation in the 5-min Na⁺ uptake in these fibroblasts (Fig. 1, Table 1). The toxin-activated influx is completely inhibited by TTX. Half-maximal inhibition is

observed at 20–40 nM TTX. The apparent affinity of TTX for the fibroblast Na⁺ channel is identical to that found in similar studies with spiking neuroblastoma cells⁵. Also, the number of Na⁺ channel receptors might be even higher in CC139 than in neuroblastoma cells, as suggested by comparison of the toxin-stimulated Na⁺ fluxes of these two cell lines (Table 1).

When Na⁺ efflux through the (Na⁺ + K⁺)ATPase system is inhibited with ouabain, the addition of veratridine (100 µM) and sea anemone toxin (10 µM) to CC139 cells leads to rapid lysis (Fig. 2), which is due to an increase in cell osmotic pressure caused by massive Na⁺ entry. Cell lysis is prevented when TTX is added before the other toxins, when ouabain is omitted (Na⁺ escapes from the cells) or when external Na⁺ is replaced by choline⁺.

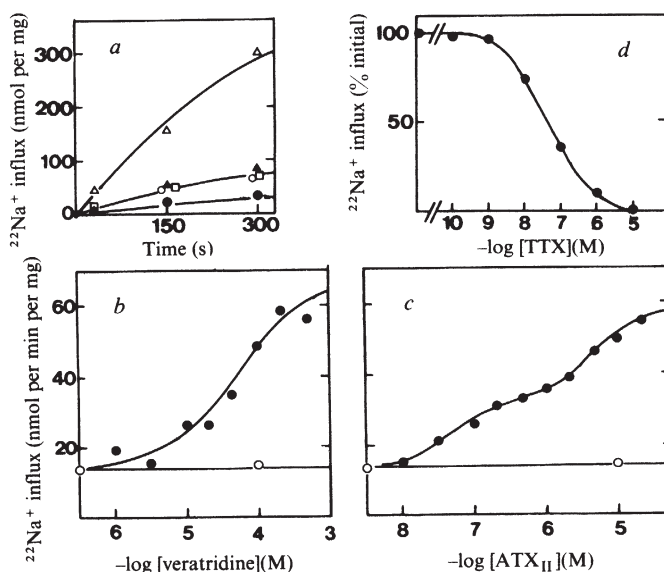


Fig. 1 Stimulation of ²²Na⁺ influx into CC139 fibroblasts by veratridine and sea anemone toxin II. Inhibition of the toxin-stimulated influx by tetrodotoxin. *a*, Kinetics of ²²Na⁺ influx into cells have been measured without toxins (●, ○) or in the presence of 10 µM sea anemone toxin II (▲), 100 µM veratridine (□) or 10 µM sea anemone toxin II + 100 µM veratridine (△). Experiments were carried out in the presence of 0.5 mM ouabain to block Na⁺ efflux catalysed by the (Na⁺ + K⁺)ATPase, except in ●. *b*, Dose-response curves for the effect of veratridine on the initial rate of ²²Na⁺ influx were determined in the absence (○) or presence of 10 µM sea anemone toxin II (●). Ouabain was present at a concentration of 0.5 mM. *c*, Dose-response curves for the effect of sea anemone toxin II (ATX_{II}) determined in the absence (○) or presence of veratridine (100 µM) (●). Ouabain was present at a concentration of 0.5 mM. *d*, Inhibition by TTX of the stimulating effect induced by the mixture of sea anemone toxin II (10 µM) and veratridine (100 µM) on the initial rate of ²²Na⁺ influx in the presence of 0.5 mM ouabain. ²²Na⁺ influx into confluent monolayers of CC139 cells was determined at 37 °C essentially as described previously for neuroblastoma cells⁵: culture medium of 35-mm dishes was first replaced by 1 ml of a saline solution buffered with 25 mM HEPES-Tris (pH 7.4) and containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM Mg SO₄, 5 mM glucose and 0.1 mg ml⁻¹ bovine serum albumin. After a 5-min preincubation of cells in this medium, ²²Na⁺ influx was then initiated by replacing the preincubation medium by 1 ml of the same saline solution isotopically labelled with ²²NaCl (2 µCi ml⁻¹). Ouabain (0.5 mM) and toxins were present in this uptake medium except when indicated. ²²Na⁺ influx was stopped 5 min later by removing the radioactive medium and washing the cells three times with 1 ml of a Na⁺- and K⁺-free saline solution at room temperature. In these conditions less than 5% of intracellular ²²Na⁺ is lost during the 15 s of the washing procedure. After solubilization of cells in 1 ml of NaOH (0.1 M), radioactivity was determined by scintillation counting and the initial rate of ²²Na⁺ influx calculated from the amount of ²²Na⁺ taken up in 5 min assuming linear kinetics. The time points of *a* were determined as described for the 5-min uptake points.

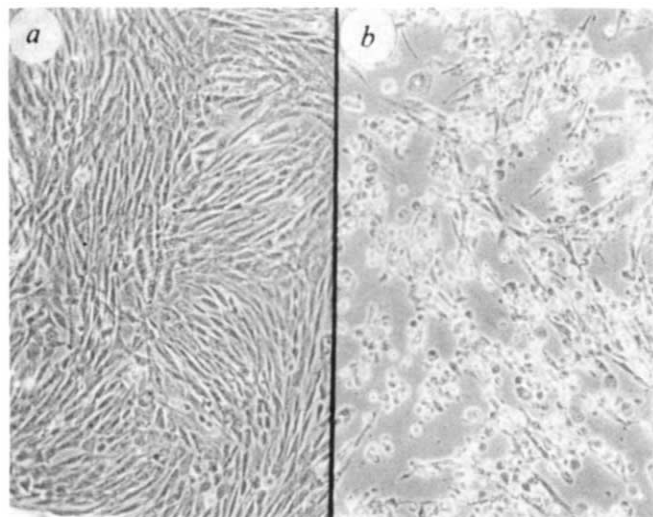


Fig. 2 Veratridine and sea anemone toxin-induced cell lysis in CC139 fibroblasts. Cells cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum were incubated with no drug (a) or with 100 μ M veratridine + 10 μ M sea anemone toxin and 0.5 mM ouabain (b). Phase contrast micrographs were taken 12 h after drug addition. Cell swelling is seen only after at least 30 min and 1 h before cell lysis (few cells) is detected. However, 2–3 h are required to see massive cell lysis. Incubation of the cells with ouabain alone or with the toxin mixture in the absence of ouabain over 24 h does not induce cell lysis (not shown).

Although CC139 cells have the morphology of fibroblasts, form fibrosarcoma tumours and do not generate action potentials, the origin of this established cell line cannot be known definitively. We therefore looked for fast Na^+ channels in two other fibroblast lines of Chinese hamster (CHO and CHL-V79), in a rat Fisher fibroblast line⁷ and its polyoma virus-transformed

derivative, and in chick embryo fibroblasts. Interestingly, all the cells possess a Na^+ channel stimulated by the mixture of veratridine and sea anemone toxins (Table 1). Although the stimulation effected by these toxins varied from 1.5- to 10-fold depending on the cell line, 30–50 nM TTX elicited half-maximal inhibition of Na^+ influx in all the cells tested. These results demonstrate the ubiquity of the fast Na^+ channel in fibroblasts from different origins.

It has been proposed that an increase in Na^+ influx might be the initial mitogenic signal in many cell systems⁸. Therefore, we have investigated whether the fast Na^+ channel of CC139 fibroblasts has any role in controlling their division. These cells can be arrested in G_0 by serum depletion. Figure 3 shows that reinitiation of DNA synthesis by 10% fetal calf serum is preceded by a very early threefold stimulation of Na^+ influx. The influx in these conditions is not blocked by TTX. This result, together with the observations that TTX does not affect the Na^+ uptake of growing fibroblasts, prevent reinitiation of DNA synthesis or alter cell division (not shown), strongly suggests that the TTX-sensitive Na^+ channel of fibroblasts is not involved in growth control.

Accordingly, these fibroblasts, like excitable cells, have receptors for veratridine, sea anemone toxin and TTX. The dose-response curves for the association of the different toxins with their respective receptors occur in a range of toxin concentrations which is similar for fibroblasts (Fig. 1b, c, d) and for excitable cells such as neuroblastoma⁵. Veratridine or sea anemone toxin alone significantly stimulate $^{22}\text{Na}^+$ entry through the TTX-sensitive Na^+ channel of neuroblastoma cells, and the two toxins act in synergy. In contrast, neither toxin alone stimulates $^{22}\text{Na}^+$ entry into fibroblasts (Fig. 1a); the mixture of the two toxins is essential for activation of the TTX-sensitive Na^+ channel. Electrophysiological studies are under way in an attempt to understand the differences between TTX-sensitive Na^+ channels in fibroblasts and those in excitable cells like neuroblastoma cells. Also, we hope to use the toxin-activable Na^+ channel of CC139 fibroblasts as a Na^+ microinjection device with which to

Table 1 Effect of veratridine and sea anemone toxin II on Na^+ influx in various fibroblast cell types

Cell type	Origin	5 min $^{22}\text{Na}^+$ uptake (nmol per mg protein)		
		Basal	Veratridine + sea anemone toxin	Stimulation (fold)
CC139	Chinese hamster lung fibroblast (ATCC)	72	570	7.9
CHL-V79	Chinese hamster lung fibroblast (ATCC)	88	95	1.1(2.1)
CHO	Chinese hamster ovary (Toronto's strain)	67	190	2.8(4.7)
FR-3T3	Rat Fisher fibroblasts ⁷	16	154	9.6
3T3-PY	Rat Fisher-polyoma transformed	67	132	2
CEF	Secondary chick embryo fibroblast	67	93	1.4
NIE-115	Neuroblastoma	46	320	7

$^{22}\text{Na}^+$ uptake was measured as described in Fig. 1 legend, in the presence of 0.5 mM ouabain (basal) or 100 μ M veratridine + 10 μ M sea anemone toxin II for the toxin-stimulated influx. Values in parentheses represent the toxin-stimulation factor when cells were arrested by serum deprivation (serum deprivation reduces basal Na^+ influx, see Fig. 3). All values represent the average of duplicate experiments (less than 10% difference was observed in duplicate determinations).

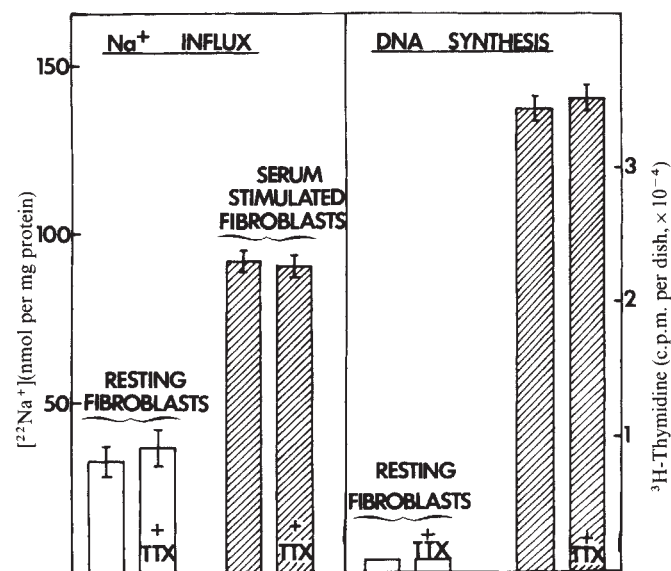


Fig. 3 Serum induced Na^+ influx and DNA synthesis in quiescent fibroblasts. Confluent CC139 fibroblasts were arrested in G_0/G_1 by incubating the cells in serum-free medium for 30 h. Na^+ influx was measured in the presence of 0.5 mM ouabain. Values represent 5 min $^{22}\text{Na}^+$ uptake in resting fibroblasts or in cells stimulated for 5 min with 10% fetal calf serum (hatched bar). For measurements of DNA synthesis, serum-deprived cells were incubated for 24 h in the presence of 0.5 μ Ci ^3H -thymidine without (empty bar) or with (hatched bar) 10% fetal calf serum. Where indicated + TTX, tetrodotoxin was added at 10^{-5} M during Na^+ uptake studies or during the 24 h of thymidine incorporation.

analyse the possible role of increased Na^+ influx in mitogenicity. These cells respond well to purified growth factors⁹. Finally, the toxin-effected cell lysis shown in Fig. 2 provides a powerful selection procedure for developing the genetics of Na^+ gating membrane components in a diploid and rapidly growing cell line.

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Loss of immune competence with age may be due to auto-anti-idiotypic antibody regulation

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The decline in immune function with ageing involves both a loss of B-cell function^{1,2} and a decline in T-cell function³⁻⁵. This picture has been complicated by the recent observation that normal old mice produce more B-cell mitogen-induced autoantibodies to syngeneic erythrocytes than do young animals⁶. They also produce elevated levels of IgM and IgA anti-IgG autoantibodies⁷ and increased suppressor-cell activities⁸⁻¹⁰. It is therefore difficult to explain the relationships between autoantibody production, increased suppressor-cell activities and the loss of immune competence in old animals. Recently, auto-anti-idiotypic antibody regulation has been proposed to explain a rapid decline in the antibody-forming cell response to antigen during a normal immune response in young adult mice¹¹. Schrater *et al.*¹² have suggested that this antibody, produced during the immune response, combines with B-cell-surface antigen receptors and thus inhibits antibody secretion. If the number of B cells is normal in old animals¹³, but their function is impaired, it may be that the decline in immune competence with age is due to auto-anti-idiotypic antibody regulation, perhaps affecting the B-cell-surface antigen receptors and preventing the cell from responding normally to exogenous antigens. We have therefore investigated whether auto-anti-idiotypic antibody could be detected in immune splenic B cells from old and young mice of the same strain. We report here that auto-anti-idiotypic antibody regulation seems to have a role in the loss of B-cell function with age.

We first immunized various aged C57BL/6J male mice intraperitoneally (i.p.) with a highly thymic-dependent antigen, trinitrophenylated bovine γ -globulin (TNP-BGG) in complete Freund's adjuvant (CFA). Splenic anti-TNP plaque-forming cell (PFC) responses were assayed 2 weeks after immunization. As shown in Table 1, 4-month-old mice produced high numbers of IgM, IgG and IgA anti-TNP PFCs whereas the 1- and 2-month-old mice produced relatively low numbers. However, mice of 8 months or older showed a significant decline in the number of anti-TNP PFCs in the three isotypes studied as compared with the PFC responses of the 4-month-old group. Thus, aged ani-

Table 1 Effect of age on the number of splenic anti-TNP PFCs in C57BL/6J mice given TNP-BGG

Age (months)	No. of mice	Splenic anti-TNP PFCs per 10^8 cells		
		IgM PFCs	IgG PFCs	IgA PFCs
1	13	1,090 (± 0.56) $P < 0.001$	1,594 (± 0.83) NS	559 (± 0.65) $P < 0.001$
2	26	1,146 (± 0.91) $P < 0.005$	1,464 (± 0.58) $P < 0.02$	1,479 (± 0.62) $P < 0.001$
4	19	2,325 (± 0.48)	2,323 (± 0.55)	3,153 (± 0.42)
8	25	1,978 (± 0.39) NS	1,266 (± 0.35) $P < 0.001$	1,288 (± 0.59) $P < 0.001$
11	3	1,807 (± 0.39) NS	1,024 (± 0.57) $P < 0.05$	1,499 (± 0.37) $P < 0.01$
15	5	707	987	833
24	2	351 (± 0.40) $P < 0.001$	337 (± 1.10) $P < 0.001$	456 (± 1.62) $P < 0.001$

Male mice received 500 μg TNP-BGG in CFA i.p. Splenic anti-TNP PFCs per 10^8 nucleated cells were assayed 2 weeks after immunization. Data presented are the geometric means of pooled spleens from three to five animals from three to six independent experiments. The data from the 11- and 24-month groups are geometric means from individual spleens. The data from the 15-month group are from pooled spleens from five animals from one experiment. The value in parentheses is the natural logarithm of s.d. obtained from natural log-transformed data. The *t*-test was used to evaluate the statistical significance of differences on natural long-transformed data. NS, Not significantly different. Anti-TNP PFCs were determined by the method of Jerne *et al.*²¹ as modified for a slide assay by Dresser and Greaves²². Rabbit anti-mouse IgG and rabbit anti-mouse IgA were used at predetermined optimal dilution (1:300), to develop indirect IgG and IgA plaques, respectively. The IgA-developing serum was prepared in rabbits by using pure mouse myeloma MOPC 315 (IgA λ 2). The antiserum was depleted of anti-light-chain activity by passage through a pure mouse IgG-Sepharose 4-B immunoabsorbent column. The IgG-developing serum was prepared in rabbits by using purified mouse IgG (Miles, Lot 51). The antisera showed only a single line against normal mouse sera and pure IgG on Ouchterlony analysis. Pure mouse IgA or IgG when added to the PFC assay inhibited the development of only IgA or IgG PFCs, respectively, when the respective developing sera were added. Before developing indirect plaques, goat anti- μ was used to inhibit >95% IgM plaques during the first incubation period. The anti- μ antiserum was prepared in goats by using pure mouse MOPC 104E (IgM λ 1) (Bionetics, Lot AJ110). It was passaged through a pure mouse IgG-Sepharose 4-B immunoabsorbent column to deplete anti-light-chain activity.

Table 2 Effect of age on the heterogeneity of affinity of splenic anti-TNP PFC responses in C57BL/6J mice

Age (months)	No. of mice	Heterogeneity index		
		For IgM PFCs	For IgG PFCs	For IgA PFCs
2	15	0.88 $\pm$ 0.52 ($P < 0.001$)	2.27 $\pm$ 0.13 ($P < 0.02$)	1.50 $\pm$ 0.18 ($P < 0.001$)
4	12	1.64 $\pm$ 0.49	2.04 $\pm$ 0.27	2.12 $\pm$ 0.20
8	20	0.51 $\pm$ 0.38 ($P < 0.001$)	1.22 $\pm$ 0.83 ($P < 0.001$)	0.63 $\pm$ 0.63 ($P < 0.001$)
15	5	0.33	0.50	0.37
24	2	0	0	0.87 $\pm$ 0.62 ($P < 0.001$)

Male mice received 500 μg TNP-BGG in CFA i.p. Splenic anti-TNP PFCs were assayed 2 weeks after immunization. The Shannon heterogeneity index¹⁵ was used to describe the degree of heterogeneity of affinity of the IgM, IgG and IgA anti-TNP PFC population of pooled spleens from three to five mice. The values presented are the means $\pm$ s.d. from three or four independent experiments. The values from the 15-month group were obtained from a single pool of five mice from one experiment. The values from the 24-month group were obtained from individual spleens and presented as means $\pm$ s.d. from two animals. The larger the index, the greater the heterogeneity. The *t*-test was used to evaluate the statistical significance in the heterogeneity indices as compared with the 4-month-old group.

Table 3 Effect of age on the appearance of anti-idiotypic-blocked hapten-augmentable, anti-TNP PFCs during the normal immune response to TNP-BGG in C57BL/6J mice

Age (months)	No. of mice	TNP-EACA	Splenic anti-TNP PFCs per 10 ⁸ cells					
			IgM PFCs	(% aug PFCs)	IgG PFCs	(% aug PFCs)	IgA PFCs	(% aug PFCs)
2	15	—	2,154		2,035		1,838	
		+	2,283	6%	2,340	15%	2,022	10%
4	10	—	2,084		1,350		2,394	
		+	2,272	9% (NS)	1,755	30% ($P < 0.005$)	2,729	14% (NS)
8	15	—	3,818		1,856		2,196	
		+	5,765	51% ($P < 0.001$)	3,972	114% ($P < 0.001$)	4,085	86% ($P < 0.001$)
15	5	—	707		987		833	
24	2	+	1,308	85%	2,240	127%	1,974	137%
		—	351		337		456	
		+	1,127	221%	896	166%	880	43%

Male mice received 500 μ g TNP-BGG in CFA i.p. Splenic anti-TNP PFCs per 10⁸ nucleated cells were assayed 2 weeks after immunization. Data presented are the geometric means of 10–15 animals from two or three independent experiments. Spleen tissues were assayed as a single pool from five animals in each experiment. Spleen tissues from the 15-month-old group were assayed as a single pool from five mice in one experiment. Spleen tissues from the 24-month group were assayed as individual spleens and geometric means of two animals from one experiment are presented. Percentage augmentation of PFCs by optimal hapten concentration (1×10^{-9} – 1×10^{-7} M TNP-EACA) is calculated as: $100 \times (\text{PFCs in presence of hapten} - \text{PFCs in absence of hapten}) / \text{PFC in absence of hapten}$. The *t*-test was used to evaluate the statistical significance of differences on natural log-transformed data as compared with the 2-month group.

mals exhibited a loss of IgM, IgG and IgA PFCs in their primary immune response to TNP-BGG. These results are consistent with other data reported for this strain, with a loss of IgM anti-sheep red blood cell (SRBC) PFCs *in vitro* in mice over 4 months of age¹⁴.

We then investigated the heterogeneity of the PFC response during ageing. The distribution of PFCs with regard to avidity was determined by hapten inhibition of plaque formation in mice of different ages and can be quantitated using the Shannon function¹⁵ as heterogeneity index, thereby permitting a simple statistical evaluation of the data. This index is expressed as a log₂ term and increases with increasing heterogeneity of the PFC distribution. The heterogeneity indices calculated from these data (distributions not shown) show a significant decrease in heterogeneity of splenic PFCs with age (Table 2). Thus, IgM, IgG and IgA PFCs from 8-month-old animals are significantly ($P < 0.001$) less heterogeneous than PFCs from 4-month-old animals. The affinities of IgM and IgG PFCs from the 24-month-old animals are completely restricted but the IgA PFCs still retain some heterogeneity, although this is significantly ($P < 0.001$) less than that in the 4-month-old group. Thus, with ageing, there is a progressive decrease in heterogeneity, characterized by a loss of high-avidity PFC subpopulations in older animals.

Auto-anti-idiotypic antibodies have been shown to be responsible for a decline in the number of PFCs, a reduction in the heterogeneity of avidity of PFCs and the occurrence of hapten-augmentable PFCs (anti-idiotypic blocked)¹². We therefore investigated the effect of age on the appearance of anti-idiotypic-blocked, hapten-augmentable anti-TNP PFCs. As shown in Table 3, 8-month-old mice, during the primary immune response to TNP-BGG, produced a significantly ($P < 0.001$) high percentage of hapten-augmentable PFC responses in the IgM (51%), IgG (114%) and IgA (86%) isotypes studied as compared with the 2-month-old group, which did not show any appreciable (6–15%) hapten-augmentable PFCs. Older mice also produced a high percentage of hapten-augmentable PFC responses, ranging from 85% to 221% in the three isotypes. These data support the hypothesis that auto-anti-idiotypic antibody de-regulation of splenic anti-TNP PFC responses of old mice is responsible for the age-associated decline in the number and heterogeneity of avidity of the PFCs in the three isotypes studied.

Other studies¹⁶ describe an assay for anti-idiotypic antibody that makes use of the ability of such antibody in the immune sera to inhibit plaque formation by idiotypic-producing cells. This

Table 4 Anti-idiotypic antibody activity in immune sera from ageing C57BL/6J mice as assayed by hapten-reversible plaque inhibition

Serum source	Anti-TNP titre (reciprocal)	% Of control response					
		PFCs from spleen 1			PFCs from spleen 2		
		IgM PFCs	IgG PFCs	IgA PFCs	IgM PFCs	IgG PFCs	IgA PFCs
NMS	<2	100	100	100	100	100	100
Immune 2-month-old	80	100	110	99	78	83	95
Immune 4-month-old	80	78	52	96	72	75	96
Immune 8-month-old	160	42	23	73	31	57	71
Immune 11-month-old	80	62	33	88	53	39	50
Immune 15-month-old	160	47	40	88	52	37	60
Immune 24-month-old	160	42	23	45	ND	ND	ND

Immune spleen cells were obtained from individual 2-month-old C57BL/6J male mice that had been immunized i.p. with 500 μ g TNP-BGG in CFA 14 days before assay. The control values (PFCs determined in the presence of normal mouse serum [NMS]) ranged for 1,667 IgM, 5,451 IgG and 3,608 IgA anti-TNP PFCs per 10⁸ nucleated cells from spleen 1 and 2,073 IgM, 2,439 IgG and 10,829 IgA PFC's from spleen 2. Immune cells at $3-5 \times 10^7$ cells per ml were incubated with immune sera or NMS at a 1:10 final dilution for 5 min at 4°C. The cells were washed once, resuspended to the original volume, and plated. The inhibition of plaque formation was completely reversible by addition of hapten. The data illustrated are from one representative experiment out of six independent experiments. Anti-TNP titre was determined by passive hemagglutination. ND, Not determined.

The NMS pool was obtained from 10 C57BL/6J male mice at 10 weeks of age. Pools of immune sera were obtained from groups of 3–10 C57BL/6J male mice at various indicated ages, which had been immunized with 500 μ g TNP-BGG in CFA i.p. 14 days before collection of sera.

inhibition was reversible by the hapten, TNP- ϵ -amino-*n*-caproic acid (EACA). Results in Table 4 show that pools of immune sera from 8-month or older mice immunized 14 days previously with TNP-BGG in CFA caused a hapten-reversible block of plaque formation by spleen cells from TNP-BGG immune 2-month-old mice. In contrast, immune sera from 2-month-old mice failed to inhibit the PFC response.

The above findings suggest that the loss of immune competence in the splenic B-cell population may be due to auto-anti-idiotypic antibody regulation. The nature of the stimulus for the production of auto-anti-idiotypic antibody with age is unknown. However, the reported¹³ increased appearance of new or altered antigenic determinants on the surface membrane of lymphoid cells from old mice or possibly alterations in I-region products with age¹⁷, may serve as an attractive model to explain the increased appearance of auto-anti-idiotypic reactive B-cell clones. Also, auto-anti-idiotypic antibody may contribute to the increased suppressor cell activities in old

animals. This is supported by the findings that anti-idiotypic antibody can induce the activation of suppressor T cells in normal young adult mice¹⁸⁻²⁰. This type of observation has not been demonstrated in old animals. Furthermore, the relationship between auto-anti-idiotypic antibody regulation in old animals and auto-immune disease remains unclear.

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Phenotypic changes of phytohaemagglutinin-stimulated hairy cells

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Hairy cell leukaemia (leukaemic reticuloendotheliosis) appears to be a homogeneous and well defined disease on the basis of clinical presentation, light and electron microscopic features and cytochemical characteristics¹, but the study of immunological markers of hairy cells (HC) from many patients reveals apparent heterogeneity. The most common phenotype associates B-cell and monocytic properties: HC usually express monoclonal surface (and in certain cases cytoplasmic) immunoglobulins, receptors for IgM and IgG Fc, and mouse erythrocytes, and Ia-like antigens. Additionally, they are capable of phagocytosis, glass adherence, lysozyme and peroxidase synthesis²⁻¹⁸. However, most of these features are not constant and cases have been reported in which HC fail to express one or more of these properties. In certain cases HC even display a T-cell phenotype, while, in others, features of both T and B cells are expressed^{16,19-22}. Moreover, in two recently studied patients, the phenotype of HC in the blood differed from that in the spleen (B + T in the blood and B in the spleen)¹⁶. These surprising discrepancies led us to hypothesize that HC from the same individual might be able to express different phenotypes following an appropriate stimulus. We therefore studied immunological parameters of HC stimulated by mitogens and the results indeed showed that after stimulation by phytohaemagglutinin (PHA) the cells switched from B to T or B + T phenotypes.

The three patients studied were affected with clinically and morphologically typical HC leukaemia. They had not previously received specific therapy. In patient Go., HC were obtained twice (3 months apart) from leukaemic blood. On both occasions, cell suspensions resulting from Ficoll centrifugation contained 10% lymphocytes and 90% HC as shown by cytol-

Table 1 Immunological markers of fresh and cultured hairy cells (% positive cells)

Patients	Cells studied	SIg	cIg	γ FcR	E rosettes	Anti-T serum
Go.	Fresh cells	77 δ λ	0	89	8	ND
	Day 4 cultured cells					
	Control	90 δ λ	56 μ λ	67	8	20*
Go. Expt 2	PHA	0	0	12	69	61*
	Fresh cells	71 δ λ	82 μ λ	73	37	25
	Day 3 cultured cells					
Cha.	Control	69 δ λ	82 μ λ	69	10	12
	PHA	0	0	2	80	82
	Fresh cells	38 γ κ (μ δ)	0	1	1.5	1
Que.	Day 14 cultured cells					
	Control	90 γ κ (μ δ)	0	51	4	5
	PHA	0.5	0	0	83	92
Que.	Fresh cells	95 δ λ (μ)	67 μ λ	90	6	5
	Day 14 cultured cells					
	Control	83 δ λ (μ)	57 μ λ	99	1	3
	PHA	2	50 μ λ	96	88	90

The cells obtained from blood or spleen were studied for cytological (Giemsa staining and tartrate-resistant acid phosphatase activity) and immunological parameters, then cultured at 1×10^6 cells ml⁻¹ in RPMI 1640 medium containing 10% fetal calf serum (Gibco) either with or without PHA (PHA M, Gibco, final concentration 0.6%). Cultured cells were repeatedly studied for cytology, cytochemistry, cell numeration, thymidine incorporation and immunological features. Surface and cytoplasmic immunoglobulins (SIg and cIg) were assessed by direct immunofluorescence with rhodamine-conjugated rabbit F(ab')₂ fragments prepared in our laboratory as previously described²³. Conjugates polyvalent for human Ig and monospecific for μ , γ , σ , α , κ and λ chains were used. Ig Fc receptors (γ Fc R) were detected by immunofluorescence with immune complexes in conditions in which the positive cells are predominantly B cells²⁴. E-rosette forming cells were enumerated according to a classical method²⁵. Human T-lymphocyte specific antigens were studied by indirect immunofluorescence with a rabbit antiserum raised against peripheral T cells and a rhodamine-coupled sheep F(ab')₂ fragment anti-rabbit IgG (ref. 26). ND, Not determined.

* Performed on day 7, when the control and PHA-stimulated cultures contained 26% and 81% E-rosetting cells respectively.

gical and cytochemical examination. Cells obtained from the spleen (splenectomy) were almost all HC in patient Cha. and 95% HC in patient Que. Cells from both blood and spleen were studied for lymphocyte markers and cultured with or without PHA; cultured cells were serially examined for cell number, tritiated thymidine incorporation, cytology and lymphocyte markers (see legend to Table 1).

In the first study of cells from patient Go., the fresh HC displayed a B-lymphocyte phenotype with δ λ monotypic SIg (5–20% of the cells also stained for μ , γ and κ , which probably

corresponded to exogenous Ig bound to Fc receptors since only δ and λ chains were subsequently found on cultured cells), γ FcR and no T-cell markers (Table 1). PHA-stimulated cells were not examined before day 4 of culture. At this time, more than 90% of the cells in both PHA-stimulated and control cultures were typical HC on morphological grounds. In the control culture, the B phenotype remained stable except that monotypic cIg (IgM λ) became detectable. On the contrary, PHA-stimulated HC had lost SIg and γ FcR, formed E rosettes and were strongly stained by anti-T serum, thus displaying a T-lymphocyte phenotype. The immunological phenotype in both control and PHA-stimulated cultures remained the same for the 5 following days, then most PHA-stimulated cells died. During this period, the appearance of PHA-stimulated cells changed: 50% of cells on day 5 and 60–70% the following days were large blast cells showing a strong acid phosphatase positivity which was tartrate-sensitive. Cell numbers were relatively stable and roughly similar in control and stimulated cultures. Relatively low levels of thymidine incorporation were found with stimulation indices of 18.6 and 3.0 on days 4 and 6 after PHA-stimulation (similar observations were made in patients Cha. and Que. with stimulation indices varying between 7.5 and 9.7 in PHA-stimulated cultures on days 4 and 6). These data and the rapidity (24 h) of morphological changes suggest that the T-blast cells may be derived from the PHA-stimulated HC.

When studied again 3 months later, fresh HC from patient Go.'s blood showed two features different from those found at first examination: most HC contained cytoplasmic μ and λ chains and a fair number had T-cell markers. Since 90% of the cells were HC, the obvious overlap of figures for T and B markers (Table 1) indicates that some HC had a mixed B + T phenotype. This mixed phenotype was lost in the control cultures (Table 1 and Fig. 1a) where HC retained B-cell characteristics only (the cells staining with the anti-T serum on days 3 and 6 were small lymphocytes by phase contrast microscopy). Daily study of PHA-stimulated cells showed a rapid loss of B-cell markers and the acquirement of T markers (Fig. 1b and Table 1). Morphological changes similar to those initially found were observed; the cells had a typical HC appearance till day 4 and were large blast cells on the following days.

Only 38% of fresh HC in patient Cha.'s spleen had monotypic SIg($\gamma\kappa$). Between 6 and 10% of the cells also stained for μ and δ , respectively. All these Ig chains were probably synthesized by the cells since they were found in control cultures at repeated examinations over 3 weeks. In non-stimulated cultures, we observed a progressive increase in the number of SIg-bearing cells (up to 90% on day 14) and the appearance of γ FcR which was not detectable on fresh cells (Table 1). The changes occurring after PHA-stimulation had a slower kinetics than in patient Go. On day 5, the cells had lost SIg expression but only 10%

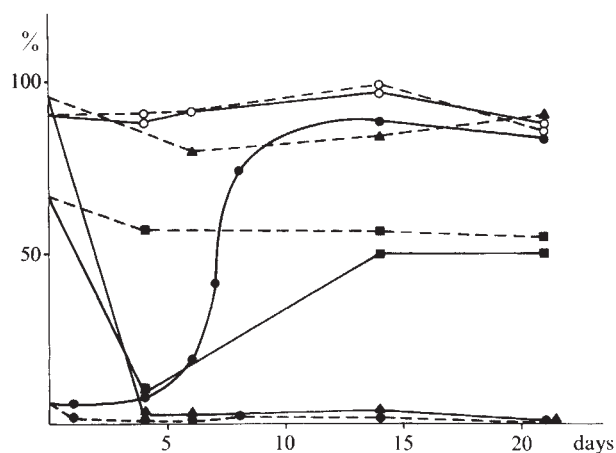


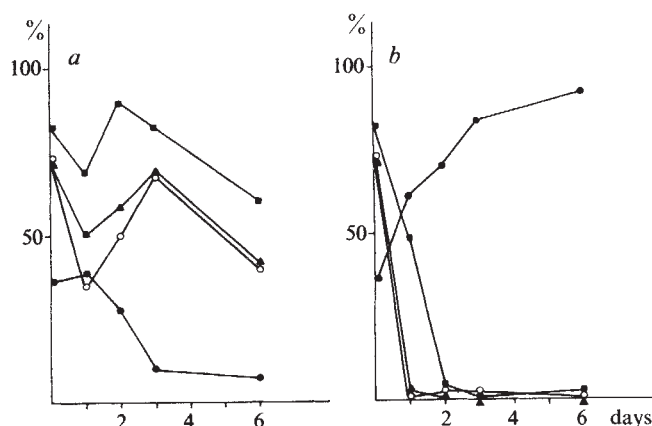
Fig. 2 B- and T-cell markers of patient Que.'s spleen cells in control (dotted lines) and PHA-stimulated (solid lines) cultures. ●, E rosettes; ▲, SIg; ■, cIg; ○, γ FcR.

formed E rosettes. These cells were virtually all HC by morphological criteria. Then, the percentage of E-rosetting cells increased. After day 12, most cells displayed T-cell features (Table 1). However, these cells were blast cells lacking cytological and cytochemical characteristics of HC. PHA-stimulated cells could not be studied after day 15 due to cell death.

Study of patient Que.'s fresh spleen HC showed a B phenotype with SIg ($\delta\lambda$ on most cells and also μ on some), cIg($\mu\lambda$) and γ FcR (Table 1). A faint surface staining and a globular cytoplasmic staining for γ , μ , κ and λ was additionally found and it was shown to be due to adsorption and pinocytosis of exogenous Ig. The B-cell phenotype remained stable in the control culture (Table 1 and Fig. 2, dotted lines). After PHA-stimulation, SIg rapidly disappeared whereas the number of cells with γ FcR was constant. After an initial drop, the number of cells containing cytoplasmic μ and λ chains returned to pre-culture levels. The number of cells forming E rosettes and staining with anti-T serum sharply increased from day 4 to day 8 and then the phenotype of PHA-stimulated cells was unchanged until day 21 (Table 1 and Fig. 2, solid lines). Further study was not possible due to cell death. During these phenotypic changes and in contrast with the first cases, stimulated HC retained their typical morphological features. Therefore, the origin of these cells with a mixed T-B phenotype from the HC in this patient was supported by morphology and by the retention of some of the original B-cell markers (especially monoclonal cytoplasmic μ and λ chains).

This study provides three major findings: (1) when HC from the same patient were studied twice, their properties were not the same; (2) spontaneous variations were observed in control cultures: acquirement of cIg or of γ FcR, switch from a B + T to a B phenotype. These data are in agreement with the finding of a different phenotype in the spleen and the blood from two patients¹⁶. Moreover, sequential studies of immunological markers in the same patients showed clear-cut fluctuations, as reported very recently²⁷; (3) the most striking finding in our study is that PHA stimulation induced a switch from a B (patients Go. and Cha.) or a B + T (patient Go.) phenotype to a T phenotype, or from a B to a B + T phenotype (patient Que.). The two T-cell markers found on these cells were distinct since we were unable to inhibit E-rosette formation by preincubation with anti-T serum. Cells binding sheep erythrocytes were actual E rosettes and not immune rosettes which could have resulted from secretion or surface expression of Ig with anti-sheep red cell activity, as sometimes observed in CLL²⁸. Indeed, rosetting was obtained only in the thermal and incubation conditions needed for T cells; it was not inhibited by incubation with anti-Ig sera and the cells did not form plaques with sheep erythrocytes. It must be stressed that these E rosettes were centered by lymphoid cells (as shown by phase contrast microscopy and

Fig. 1 B and T cell markers of patient Go.'s blood cells cultured a, without or b, with PHA (second experiment). ●, E rosettes; ▲, SIg; ■, cIg; ○, γ FcR. The number of cells staining with the anti-T serum paralleled that of E rosettes (not shown).



Giemsa staining of cytocentrifuge slides) and were not just clumps of red cells due to PHA in the medium (which was indeed a problem during the 2 or 3 first days of culture although HC were washed several times before rosette assay).

In two cases, there is no doubt that the cells which showed T-cell properties after PHA-stimulation did not result from the expansion of normal T cells and were derived from the original HC since: (1) These cells were still typical HC by morphological and cytochemical criteria. (2) In patient Que., they continued to express γ FcR and the same intracytoplasmic Ig chains as the fresh cells. (3) In patient Go., the changes were too rapid (already clear-cut after 1 day of culture) to have been accountable by the growth of normal T cells. (4) Low levels of mitogenic stimulation and roughly stable or slightly decreased cell numbers similarly negate this hypothesis. In patient Cha., the cells with T-cell markers on day 12 of culture which could not be identified as HC upon morphological grounds were nevertheless probably derived from HC. Indeed, day 5 PHA-stimulated cultures from this patient's cells contain exclusively typical HC which had lost SIg while 10% of them had become E positive. The morphological changes subsequently observed are probably similar to those observed in patient Go., in whom typical HC with T-cell properties were replaced by blast cells probably derived from them (since this morphological change was observed within 24 h).

Since HC have peculiar surface properties, one could hypothesize that T-cell markers in PHA-stimulated cultures may not be endogenous but result from adsorption of membrane molecules released in the culture medium by PHA-stimulated normal T cells. This hypothesis may be ruled out for the following reasons: (1) cells from control cultures were incubated for 1 h, 3 h and overnight at 37°C in supernatant media of PHA-stimulated cultures containing mostly cells with T markers. This incubation did not result in any change of the phenotype of these cells. (2) On several occasions, in patient Go., control and pokeweed-mitogen stimulated cultures contained a fair number of T cells (10–30%, with a similar percentage of large blast cells on Giemsa-stained smears) whereas HC in these cultures had no T-cell markers; (3) This hypothesis may hardly explain the loss of B cell features.

It is therefore clear that PHA actually induces major phenotypic changes of HC. This phenomenon is reversible since, when cultured cells with newly acquired T cell features were washed several times to remove the bulk of PHA and incubated in fresh medium without PHA, they lost T-cell characteristics and expressed the same B-cell properties as before culture within 2 days. Preliminary results of studies performed with other mitogens showed that concanavalin A induced changes similar to those observed with PHA, whereas we observed a plasmacytic differentiation after stimulation with the mitogen from *nocardia opaca*. If similar changes occur *in vivo*, our observation reconciliates the published discrepancies in immunological phenotypes and HC may be considered as very remarkable cells capable of expressing different properties after an appropriate stimulation.

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The immunoglobulin μ constant region gene is expressed in mouse thymocytes

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It has been a matter of controversy whether the functional capacity of T cells to discriminate between antigens is mediated via immunoglobulin, an immunoglobulin-like molecule, or by the product(s) of unrelated genes. The progenitors of immunoglobulin-secreting cells, B cells, express membrane-bound immunoglobulin as the antigen-specific receptor on their surface¹. For T cells, although products of immunoglobulin heavy chain variable region genes are implicated as receptor components^{2–4}, there has been no compelling immunochemical evidence for participation of either immunoglobulin light chains or heavy chain constant regions (see refs 2–6 for the disparate views). Recently, using cloned immunoglobulin DNA sequences as hybridization probes, we have demonstrated that the immunoglobulin C_{μ} gene, but not the C_{κ} gene, is expressed as polyadenylated RNA in some T cell tumour (T lymphoma) cell lines⁷. Individual T lymphoma lines yielded up to three discrete μ RNA species of different sizes (1.9, 2.2 and 3.0 kilobases), each species being different in size from the major μ RNA species present in B lymphoma cells (2.4 and 2.7 kilobases)^{7,8}. We show here that cells from the normal mouse thymus contain μ RNA species, indistinguishable in size from those in T lymphoma cells, but contain little if any κ RNA.

To detect μ and κ RNAs in T- and B-cell RNA preparations, we used as probes DNA fragments⁷ derived from plasmids bearing cDNA copies of a μ (ref. 9) and a κ (ref. 10) mRNA. Polyadenylated RNA from the cells was fractionated by agarose gel electrophoresis, blotted from the gel onto diazobenzyloxymethyl paper and hybridized with the ³²P-DNA probes¹¹. RNA species which hybridized were detected by autoradiography. Thymocytes were prepared¹² from 5–6-week-old male CBA mice with care to exclude parathymic lymph nodes. They were found to be contaminated by 0.2–0.3% of surface immunoglobulin-positive cells, presumably B cells. The contaminating B cells were depleted at least threefold by treatment of the thymocytes with antiserum against Ia^k followed by rabbit complement¹³. To evaluate further whether contaminating B cells might contribute to immunoglobulin RNA in thymocyte RNA preparations, we characterized RNA obtained from three sources of B cells: normal spleen, spleen cells from

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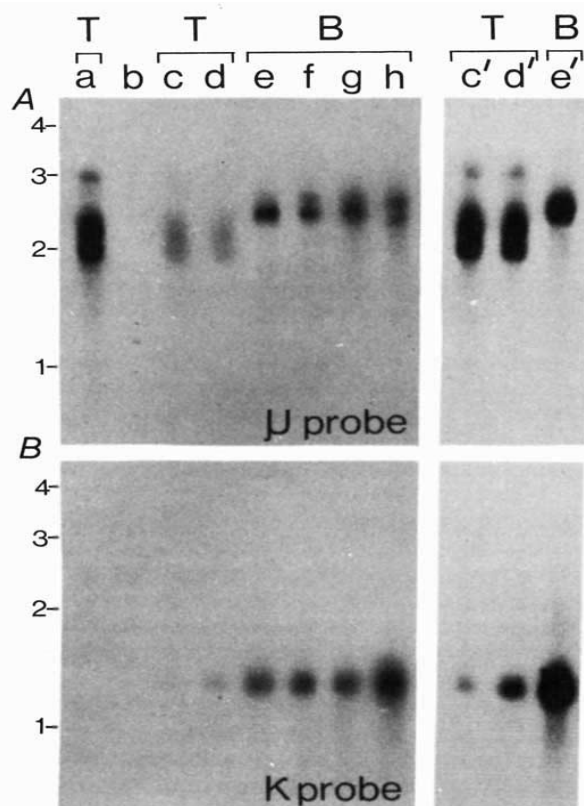


Fig. 1 Detection of A, μ RNA; and B, κ RNA in T and B cells. Polyadenylated RNA isolated from the cells indicated was denatured, fractionated by agarose gel electrophoresis, transferred to diazobenzyloxymethyl paper¹¹, hybridized with ³²P-DNA from cloned μ (ref. 9) and κ (ref. 10) cDNAs and autoradiographed for 3 days (tracks a-h) or 10 days (tracks c', d', e'), all as described previously⁷. Polyadenylated RNA (5 μ g) was loaded onto tracks a-d and 0.5 μ g onto tracks e-h. The sizes (in kilobases) are indicated on the left. The cells used for RNA preparation were: lane a, STRij-4-2.2 T lymphoma cells⁷; lane b, EMT6 sarcoma cells⁷; lanes c, d, c' and d', thymus cells prepared from 5-6 week old male CBA/CaHWehi mice. The cells used for the RNA preparation in lanes c and c' had been treated¹³ with antiserum against Ia^k (prepared in the congenic A.TH anti-A.TL combination) followed by rabbit complement treatment and removal of damaged cells¹². Anti-Ia^k/complement treatment reduced the number of surface immunoglobulin-positive cells (determined by immunofluorescence) from 0.2% to <0.06% and lowered the level of functional B cells detected in a limit dilution assay¹³ by at least 10-fold; lanes e, e', spleen cells, prepared from CBA.nu mice; lanes f and g, spleen cells, prepared from DBA/2 mice. The spleen cells used for lane f had been treated with anti-Thy-1 plus complement followed by damaged-cell removal, a procedure which removed 29% of the cells and all detectable T cells; lane h, W231.1 B lymphoma cells.

athymic nude mice and spleen cells treated with anti-Thy 1 plus complement to remove T cells.

Autoradiographs revealing species of RNA hybridizing with radioactive μ and κ probes are shown in Fig. 1. Relative contents of μ and κ RNA estimated from densitometric measurements on the autoradiographs are listed in Table 1.

In thymocyte RNA we detected μ RNA species of 1.9, 2.2 and 3.0 kilobases (Fig. 1a, tracks c, d, c' and d'). They were of the same size and relative abundance as μ RNA species from STRij-4 T lymphoma cells (track a). In contrast, each of the three different types of B cell preparations tested contained μ RNA species of 2.4 and 2.7 kilobases, analogous to the two species from WEHI-231 B lymphoma cells (tracks e-h and e'). As expected⁷, the control RNA from the sarcoma cell line EMT6 (track b) contained no detectable μ RNA. In comparing

the intensity of the μ RNA signals in the various tracks shown in Fig. 1, note that 10 times more RNA from thymocytes and STRij-4 T lymphoma cells than from splenic B cells and WEHI-231 B lymphoma cells was loaded on to the gel, as the μ RNA content of thymocytes was 10- to 20-fold lower than that of B cells.

It is crucial to know whether the μ RNA species are authentic products of the C_μ gene as, for example, hybridization of an RNA species distantly related to the C_μ gene has been observed in a T lymphoma¹⁴. The results shown in Fig. 1A were obtained using a probe representing only domains 1 and 2 of the C_μ gene⁷. When a cloned segment containing only C_μ domains 3 and 4 (ref. 7) was used as the probe, μ RNA species identical to those shown in Fig. 1A were detected in anti-Ia^k purified thymocytes (data not shown). Hence the thymocyte μ RNA species must each contain sequences from both the 5' and 3' halves of the C_μ gene, as is the case with T lymphoma μ RNAs⁷. We have also examined the thermal stability of hybrids formed with thymocyte RNA and the probe for domains 3 and 4. The hybrids formed with each thymocyte μ RNA species were stable to incubation at 65 °C in 0.036 M Na⁺. In these conditions, this DNA probe forms stable hybrids only with the C_μ gene¹⁵, not with any other sequence in the mouse genome. Thus the thymocyte μ RNAs are authentic products of the C_μ gene.

As κ chains comprise about 95% of the known immunoglobulin light chains in the mouse, we expected that κ RNA would be readily detectable in B cells and this was borne out (Fig. 1B, tracks e-g). κ RNA was also detectable in RNA from crude thymocyte preparations (Fig. 1B, track d), but in lower amounts than in any of the B cell preparations (Table 1). We think most of the κ RNA comes from cells of the B lineage, because it was lowered by about threefold by depletion of B cells and plasma cells from the thymocyte population (Fig. 1B, track c); the resultant molar ratio of κ RNA to μ RNA was only one tenth of that found in purified B cells (Table 1). To delineate further the cellular origins of both μ and κ RNA in thymocyte preparations, we fractionated CBA mouse thymocytes on the basis of their buoyant density by centrifugation on continuous albumin gradients. The procedure used produces a marked separation of the minor, immunologically active, low Thy-1, cortisone-resistant, medullary subset (concentrated in fraction 2, Table 1) from the major subset (mostly found in fractions 3-4)¹⁶. The few contaminating activated B cells and immunoglobulin-secreting plasma cells are concentrated in the least dense fraction¹⁷. While μ RNA of the characteristic T lymphoma size classes was found

Table 1 Relative μ and κ RNA contents of T and B cells

Cells	Purification	κ RNA/ μ RNA*
<i>Expt 1</i>		
STRij-4-2.2	Cloned cell line	<0.01
Thymus	None	0.25
Thymus	Anti-Ia ^k	0.085
Spleen	None	0.90
Spleen	Anti-Thy-1	1.00
Spleen	From athymic nude mouse	0.72
WEHI-231.1	Cloned cell line	1.83
<i>Expt 2</i>		
Thymus	None	0.065
Thymus	Fraction 1 (ρ = 1.0630-1.0730)	0.28
Thymus	Fraction 2 (ρ = 1.0730-1.0752)	0.024
Thymus	Fraction 3 (ρ = 1.0752-1.0798)	0.014
Thymus	Fraction 4 (ρ = 1.0798-1.0824)	<0.02
Thymus	Fraction 5 (ρ = 1.0824-1.0910)	<0.02
Spleen	Anti-Thy-1	1.00

* The relative amounts of μ (or κ) RNA in each sample were determined by quantitative densitometry on autoradiographs prepared as described in Fig. 1 legend. These values were normalized to the values obtained for T cell-depleted spleen cells, assuming the latter value to be 1.0 mol κ RNA per mol μ RNA.

in all density fractions with little evidence for selective concentration in any fraction, the molar ratio of κ : μ RNA decreased to ≤ 0.024 in fractions 2–5 (Table 1). More than 90% of the total κ RNA was concentrated in the least dense fraction, which contained only 12% of the thymocytes. We conclude that the κ RNA originates from contaminating plasma cells or activated B cells. Since plasma cells (as evidenced by plasmacytomas) contain about 100–1,000 times more immunoglobulin mRNA than do B cells^{7,8}, they would contain at least 40,000 times as much κ RNA as our purified thymocytes (present data). Plasma cell contamination at a level of 1 in 40,000 could be present in thymocytes and might be the origin of immunoglobulin κ chains which others have found to be synthesized by thymocyte suspensions¹⁸. Our results certainly do not confirm earlier reports^{19,20} suggesting the presence of similar amounts of κ RNA in spleen and thymus cells.

The average μ RNA content of about 5–10 molecules per thymocyte is similar to that found in some T lymphoma cell lines

that we have studied⁷. We do not know, however, what proportion of thymocytes contain μ RNA. Expression of the μ but not the κ gene in thymocytes suggests a close parallel with pre-B cells²¹ and this analogy is extended by the recently reported presence in an immature B cell lymphoma line⁸ of μ RNA species with sizes (2.1 to 3.0 kilobases), similar to those in T lymphoma lines.

Our demonstration here of C_{μ} gene expression in normal thymocytes complements our results with cloned T lymphoid cell lines⁷. Together they constitute a strong case that the C_H locus functions in at least some cells of the T lineage, perhaps to encode a component of the T cell antigen receptor.

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An unusual translocation of immunoglobulin gene segments in variants of the mouse myeloma MPC11

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Immunoglobulin light chain genes of the mouse are composed in germ-line DNA of four separate segments, the leader, V (variable), J (joining) and C (constant) segments. In immunocompetent cells a V and a J gene segment are joined by a site-specific recombination event^{1,2}. In variants of the mouse myeloma MPC11 a so-called kappa (κ) light chain fragment is expressed³ which consists of the MOPC321 leader peptide, joined to the κ constant region peptide^{4,5}. Using the Southern blotting technique⁶ we found that the gene coding for the light chain fragment has apparently been generated by an aberrant translocation of a V gene segment identical or very similar to the MOPC321 V gene segment into the large intervening sequence between the J and the C gene segments. The resulting deletion of the splice signals of the J segments could be the reason for the observed splicing between leader and C region sequences, a phenomenon which may be of general interest for the understanding of the splicing mechanism.

Two variants of myeloma MPC11 were investigated. In one only the κ light chain fragment is found; in the other one in addition an intact κ light chain with MPC11 leader and variable

region peptides is expressed. The variants are called MPC11 (Lf) and MPC11 (L + Lf), respectively. High molecular weight DNA isolated from mouse liver and the two MPC11 variants was digested with various restriction nucleases and fractionated on agarose gels. Liver DNA has been shown to contain the C gene segment in an arrangement very similar if not identical to the one found in germ-line DNA⁷. After denaturation the DNA fragments were transferred to nitrocellulose filters⁸ and hybridized with a nick-translated plasmid probe specific for the C gene segment plus about 2,000 base pairs of surrounding genomic DNA sequences. Figure 1 shows the autoradiograms obtained after digestion of the three DNAs with *EcoRI*, *SstI*, *BamHI*, and *EcoRI* plus *HpaI*. The chain length of fragments observed in these and other digestion experiments are compiled in Table 1. In the *EcoRI* digests of both, MPC11 (L + Lf) and MPC11 (Lf) DNA a 19 kilobase band was identified while liver DNA (not shown) yielded the known^{7,9,11} 15 kilobase fragment. The 19 kilobase band of MPC11 (L + Lf) is composed of two fragments of similar length as could be shown by digestion with for example *BamHI*. Two fragments were formed, the smaller of which was also found in digests of MPC11 (Lf) DNA (Fig. 1). The *BamHI* fragment of liver DNA differs from the two fragments of MPC11 DNA. A pattern of corresponding and non-corresponding fragments was also obtained in the analysis of the C region specific fragments with *HpaI* (Fig. 2). The *BamHI* and *HpaI* fragment patterns already indicate the existence of two differently rearranged κ light chain genes in MPC11 (L + Lf) DNA. Only one of the rearranged κ genes is preserved in MPC11 (Lf) DNA which must be the one responsible for the synthesis of the L chain fragment.

According to the known amino acid sequence¹⁰ of the MPC11 L chain and the nucleotide sequence of the five J gene segments^{8,9} either J1 or J2 should be the target of the site-specific recombination process which leads to the formation of the complete MPC11 L chain gene. Restriction nucleases which cleave in the large intervening sequence between J2 and the C gene segment should then generate the same fragment from MPC11 (L + Lf) and liver DNA (compare Fig. 2). This was the case in *BglII*, *XbaI*, and *SstI* digests (Figs 1, 2). The additional fragments in MPC11 (L + Lf) DNA digests correspond to the

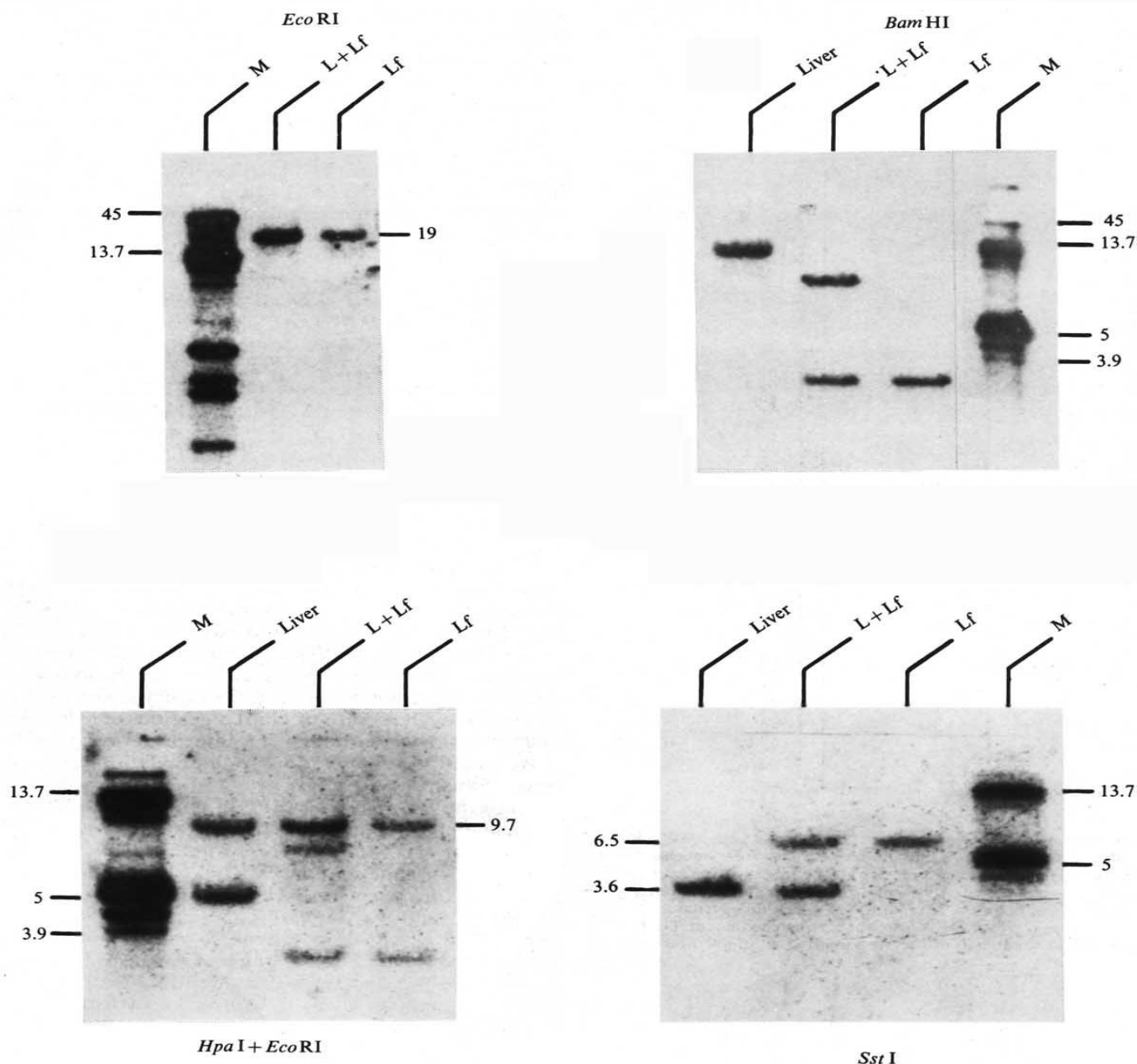


Fig. 1 Identification of DNA fragments containing the κ C gene segment in restriction endonuclease digests of mouse liver, MPC11 (L+Lf) and MPC11 (Lf) DNA. Clone 66.2 (ref. 3) designated MPC11 (L+Lf) and clone NP-2 (ref. 3) designated MPC11 (Lf), originally provided by R. Laskov (Hebrew University, Jerusalem), were maintained as solid tumours by subcutaneous transplantation in inbred BALB/c mice. DNAs were prepared according to ref. 13. Samples of 10 μ g of DNA were digested with 10 units of *EcoRI*, *BamHI*, *SstI* (Bethesda) or *HpaI* (Boehringer Mannheim) plus *EcoRI* for 3 h at 37 °C in volumes of 150–200 μ l. Plasmid DNA when mixed with mouse DNA was completely digested. DNA digests were electrophoresed on a 0.7% or 0.5% (*EcoRI* digest) agarose gel in Tris-EDTA-phosphate buffer¹⁴ containing 1 μ g ml⁻¹ ethidium bromide at 1.4 V cm⁻¹ overnight. The gels were then exposed to 254 nm light for 5–10 min¹⁵ and submitted to the transfer procedure⁶. The hybridization probe had been constructed by subcloning in pBR322 the 2.8 *HindIII*-*BamHI* fragment containing the C gene segment of the 14 kilobase *EcoRI* fragment from myeloma DNA⁷. The subcloned fragment is identical to the corresponding fragment from liver DNA (ref. 7, Fig. 2). Nick-translation^{16,17} of the plasmid DNA with [α -³²P]dATP and [α -³²P]dCTP led to 2×10^8 c.p.m. per μ g DNA. Filters which had been baked for 2 h at 80 °C *in vacuo* were preincubated in $3 \times$ SSC, $10 \times$ Denhardt's solution¹⁸ for 3 h at 68 °C and subsequently in $2 \times$ SSC, $6.6 \times$ Denhardt's solution, 50 μ g ml⁻¹ sheared and heat-denatured salmon sperm DNA, 0.1% SDS for 1 h at 68 °C¹. The filters were then incubated in a graduate in a volume of 2.5 ml of the latter solution supplemented with 40 ng ml⁻¹ heat-denatured nick-translated probe for 15 h at 68 °C under constant rotation. After this hybridization filters were washed six times for 5–10 min each in $2 \times$ SSC, $6.6 \times$ Denhardt's solution, 0.1% SDS at 68 °C and twice in $1 \times$ SSC, 0.1% SDS at 65 °C 15 min each. Some of the filters were further washed in $0.1 \times$ SSC, 0.1% SDS at 65 °C, which did not alter the band pattern shown. Dry filters were exposed to Kodak X-Omat R film for 2 d at -80 °C with a SE6 intensifying screen from Cawo (Schrobenhausen, West Germany). The slots contain the following: liver, L+Lf, Lf, restriction fragments of mouse liver DNA, MPC11 (L+Lf) and MPC11 (Lf) DNA, respectively. The hybridization marker (M) consisted of 100 pg each of a *PstI*, *HindIII*, *BamHI*, and *HpaI* digest of the genomic clone Ch4A 173/1 (ref. 7) and 100 pg of a *HindIII* plus *PstI* digest of a pBR322 subclone¹² containing the 2.7 kilobase *BglII* fragment with the C gene segment from liver DNA (compare Fig. 2). Fragment sizes are given in kilobases.

fragments in MPC11 (Lf) DNA digests, indicating that the recombination in the MPC11 (Lf) genome has occurred to the right of the *SstI* cleavage site in the large intervening sequence.

The previous conclusions are based on the assumption that the C gene segment itself and its 3' flanking sequence has not been altered. This is made likely by the occurrence of identical fragments in the digests of the three DNAs (12 kilobases in the

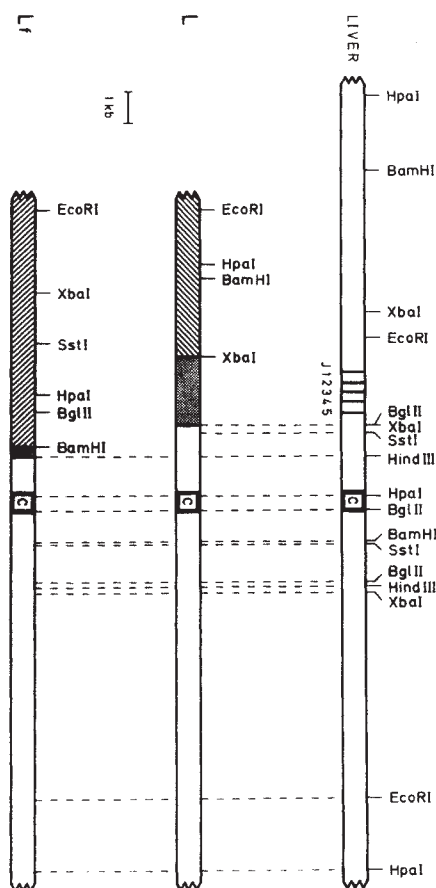


Fig. 2 Organization of the κ C gene segment in mouse liver, MPC11 (L+Lf) and MPC11 (Lf) DNA. Only those restriction endonuclease cleavage sites which were identified by Southern gel blotting experiments are shown. J (positions according to refs. 8, 9) and C gene segments are indicated by bars and boxes. Regions on the MPC11 DNA which differ from each other and from those on the liver DNA are shown as hatched boxes. DNA segments carrying the recombination site are indicated as stippled boxes.

HpaI and 2.3 kilobases in *BglII* digests) and is further supported by double digestion experiments with pairs of restriction nucleases which cleave in the C gene segment and the 3' flanking sequence and therefore should lead to the identification of the same DNA fragment in all three genomes. In *HpaI* plus *EcoRI* digests of the three DNAs the expected 9.7 kilobase fragment was found (Fig. 1). Also, *HpaI* plus *XbaI*, *BglII* plus *SstI*, and *BglII* plus *BamHI* digests yielded fragments which were common to the three DNAs (Table 1). The other fragments in the double digestion experiments are obviously derived from the 5' flanking sequences of the C gene segment.

To show directly that the recombination process leading to the formation of the Lf genome gave rise to a deletion of the J gene segments *EcoRI* and *XbaI* digests of the MPC11 DNAs were hybridized with the *EcoRI*-*BglII* fragment from liver DNA containing the J gene segments (Fig. 2; compare also refs 8, 9) which had been subcloned¹² in pBR322. No hybridization band could be detected in digests of MPC11 (Lf) DNA whereas a 19 kilobase *EcoRI* resp. a 2.2 kilobase *XbaI* fragment (Table 1), obviously derived from the chromosome containing L chain gene, was identified in digests of MPC11 (L+Lf) DNA (not shown).

The mapping data on the MPC11 L and Lf genes are summarized in Fig. 2 and compared to the arrangement of the J and C gene segments in liver DNA. From our data the region where the recombination has occurred can be confined to the *XbaI*-*BglII* fragment for the MPC11 L chain gene (in agreement with the sequence data in refs 8-10) and to the *BamHI*-*HindIII* fragment for the Lf gene (stippled boxes in Fig. 2). The *HindIII* sites to the left of the C gene segment have been found at the same positions on all three mouse DNAs. The localization of the recombination site involved in the formation of the Lf gene was supported by the results of *HinfI* digestions of MPC11 (Lf) DNA (data not shown) although the interpretation of the blots was complicated, probably due to methylation of some of the restriction sites.

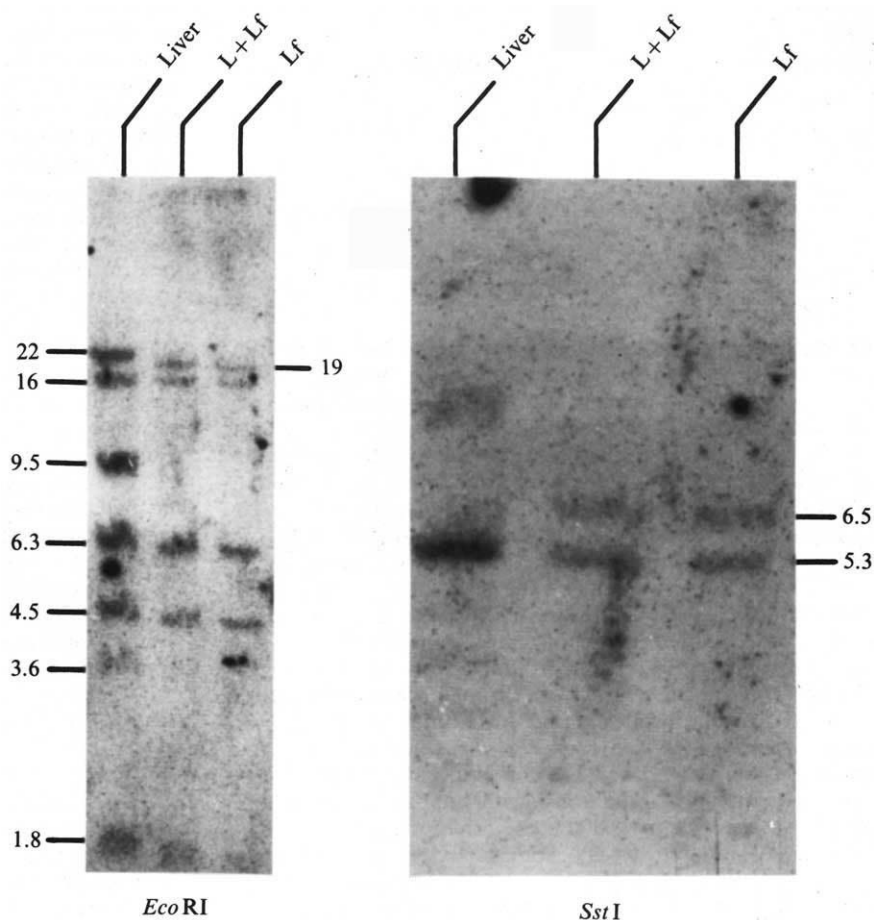
Since the Lf mRNA of MPC11 contains the MOPC321 light chain leader sequence it was of interest to search near the C gene segment of the MPC11 DNA for this sequence and also for MOPC321 V gene sequences. The hybridization probes we had available for these experiments were a *PstI* subclone of MOPC321 cDNA comprising the leader sequence and a small part of the MOPC321 V gene segment as well as a *BamHI* subclone containing only V region sequences (Fig. 3 legend). In *EcoRI* digested liver DNA we identified with either probe all DNA fragments which are known¹¹ to contain MOPC321 V region related sequences (in addition two small DNA fragments

Table 1 Length of some restriction nuclease fragments containing C or J gene segments

	LIVER			MPC11 (L+Lf)				MPC11 (Lf)	
	common	shared	unique	common	shared	L	Lf	common	unique
<i>EcoRI</i>			15			19	19		19
<i>BamHI</i>			12			8.5	3.1		3.1
<i>HpaI</i>	12		13	12		7.5	3.3	12	3.3
<i>BglII</i>	2.3	2.7		2.3	2.7		3.2	2.3	3.2
<i>SstI</i>		3.6			3.6		6.5		6.5
<i>XbaI</i>		5.4			5.4		9.7		9.7
<i>HindIII</i>	4.2			4.2				4.2	
<i>HpaI</i> + <i>EcoRI</i>	9.7		5.1	9.7		7.5	3.3	9.7	3.3
<i>HpaI</i> + <i>XbaI</i>	3.1	2.3		3.1	2.3		3.3	3.1	3.3
<i>BglII</i> + <i>SstI</i>	1.1	2.6		1.1	2.6		3.2	1.1	3.2
<i>BglII</i> + <i>BamHI</i>	1.0	2.7		1.0	2.7		2.0	1.0	2.0
<i>EcoRI</i>			15			19			None
<i>XbaI</i>			3.7			2.2			None

DNA fragments were identified by hybridization with a subclone containing the C gene segment (Fig. 1 legend), except for the last two digests where a subclone containing the J gene segments (see text) had been used. 'Common' fragments were found in all three digests and 'shared' ones only in the liver and the MPC11 (L+Lf) DNA digests; 'unique' fragments are derived exclusively from the liver, the L, or the Lf genome. Appropriate sizes are given for the fragments as they are obtained in Southern blot analysis while for the markers (Fig. 1) the more exact values are used which were determined by mapping of cloned fragments.

Fig. 3 Identification of restriction nuclease fragments in mouse liver, MPC11 (L+Lf) and MPC11 (Lf) DNA which hybridize to MOPC321 V region probe. Nuclease digestions, electrophoresis, markers, blotting, and hybridizations were as in Fig. 1, with the following exceptions: electrophoretic separation of the *Eco*RI digested DNA was for 3 d at 1 V cm^{-1} in the cold room; preincubation of the filters was in $6 \times \text{SSC}$ and hybridization in $4 \times \text{SSC}$ instead of $3 \times \text{SSC}$ and $2 \times \text{SSC}$, respectively; after hybridization the filters were washed with $1 \times \text{SSC}$ and autoradiographed for 9 d with a SE6 intensifying screen. As hybridization probes for the *Eco*RI and *Sst*I blots subcloned *Pst*I-*Pst*I and *Bam*HI-*Bam*HI fragments were used, respectively, which had been derived from a MOPC321 cDNA clone^{20,21}. The *Pst*I fragment of ~160 base pairs contains a short GC-tail, 13 base pairs of the MOPC321 5' untranslated region and the entire leader sequence plus the V region DNA sequence up to the codon for ²³Cys (I. Schechter and A. Bothwell, in preparation). The ~100 base pair *Bam*HI fragment contains part of the MOPC321 V region DNA sequence from a Gly codon at position 57, 64, or 66 to the ⁹³Gln codon^{20,21}. Very similar or identical patterns were obtained when the *Bam*HI probe was used for the *Eco*RI digest and the *Pst*I probe for the *Sst*I digest.



could be identified which are not shown in Fig. 1 of ref. 11). In the *Eco*RI digests of both MPC11 DNAs three of these fragments (22, 9.5 and 3.6 kilobases) were absent while a 19 kilobase fragment not found in the liver DNA digest was seen (Fig. 3). The absence of germ-line V gene fragments and the appearance of a new fragment indicate that MOPC321 leader and V region segments have been arranged. In the *Eco*RI, *Sst*I, and the *Xba*I digests of both MPC11 DNAs the new fragments are identical in size to the fragments detected with the C region probe in the Lf genome (Table 1); this refers to the fragments of 19 kilobases (Fig. 3), 6.5 kilobases (Fig. 3), and 9.7 kilobases (not shown), respectively. It is of particular interest that very similar or identical hybridization patterns were found with the leader and the V gene probes (Fig. 3 legend). The results are best interpreted by assuming that in the MPC11 Lf genome the V gene segment of MOPC321 or a segment with a closely related V gene has been translocated into an aberrant position inside the large intervening sequence. It is not known yet whether the sequences at this recombination point bear similarity to the ones

at or near the known J gene segments. Probably the whole region comprising the MOPC321 leader and V gene segments or a large part of this region has been translocated. Since apparently the J region with its splice points is deleted in the MPC11 (Lf) genome (see above; Fig. 2) the mRNA coding for the light chain fragment has to be formed by a splicing event (or events) in the pre-mRNA between the leader and C region. Further work is needed to establish whether the translocation of the MOPC321 gene segments into the large intervening sequence occurs directly or through a V-J joining and subsequent deletion of part of the intervening sequence including the splice signal.

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Secretion requires a cytoplasmically disposed sulphhydryl of the RER membrane

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The capacity of the rough endoplasmic reticulum (RER) membrane of eukaryotic cells to translocate nascent presecretory proteins from the cytosol to the intracisternal space is preserved on cell fractionation and can be assayed *in vitro*¹. Two attempts to characterize this translocation activity have been reported. Warren and Dobberstein² reported that microsomal membranes can be depleted of their translocation activity by extraction with a solution of high ionic strength (500 mM KCl) and that activity can be restored to the depleted membranes by re-addition of the salt extract. On the other hand, Walter *et al.*³ reported that KCl extraction of the microsomal membrane does not result in complete depletion of its translocation activity. However, mild trypsinization of the microsomal membrane released a tryptic fragment(s) from the membrane which, when recombined with a tryptically inactivated membrane fraction, restored translocation activity³. We now show that both the trypsin and the KCl extracted factors, but not the membrane-integrated remainder of the translocation apparatus, contain at least one sulphhydryl group that is essential for activity.

Our assay for the translocation activity of microsomal membranes takes advantage of the obligate coupling of translocation to proteolytic processing of nascent presecretory proteins³⁻⁵; hence, the ratio of 'processed' prolactin to 'unprocessed' preprolactin is a readily quantifiable measure of the translocation activity of the microsomal membrane.

It was previously noted⁶ that *N*-ethylmaleimide, a sulphhydryl-modifying reagent, inhibited translocation. Our data here confirm this observation. Preincubation of ribosome-stripped microsomal vesicles with 1.5 mM *N*-ethylmaleimide completely inhibited translocation, as virtually no processed prolactin was synthesized (Figs 1, 2, compare lanes 2 and 3).

A quantitative analysis of this inhibition by various concentrations of *N*-ethylmaleimide and other sulphhydryl-modifying reagents (TLCK, a trypsin inhibitor, is mildly reactive with sulphhydryl groups⁷) is shown in Fig. 3. We also analysed duplicate aliquots of these same modified samples by the signal peptidase-independent translocation assay described by Warren and Dobberstein². In this, translocation activity is determined from the ability of the microsomal membrane to protect translocated polypeptides from proteolytic digestion, as quantitated by an increase in trichloroacetic acid-precipitable radioactivity. Values comparable to those presented in Fig. 3 (data not shown) were obtained. *N*-Ethylmaleimide was the most effective inhibitor of translocation. At a concentration of less than 1.0 mM, *N*-ethylmaleimide completely inhibited translocation (Fig. 3a, b). Although the other sulphhydryl-modifying reagents tested were also effective, concentrations in excess of 1.0 mM were required to achieve complete inhibition of translocation in the given conditions. Interestingly, the bound ribosomes of 'native' rough microsomes do not protect the sensitive sulphhydryl group(s) from *N*-ethylmaleimide modification. As shown in Fig. 3b, *N*-ethylmaleimide-modified rough microsomes, when stripped of ribosomes by EDTA extraction and subsequently assayed for translocation activity, are inhibited to the same extent as an equivalent amount of microsomes which had been stripped of ribosomes before *N*-ethylmaleimide modification.

To rule out the possibility that the observed inhibition of the synthesis of processed prolactin could have resulted from an inactivation of signal peptidase rather than an impairment of translocation, *N*-ethylmaleimide-modified stripped microsomes were dissociated with sodium deoxycholate and assayed for signal peptidase activity in a post-translational assay^{8,9}. The results (Fig. 4) demonstrate that signal peptidase is not inactivated by *N*-ethylmaleimide modification.

The fact that the translocation apparatus can be separated into soluble and membrane-associated fragments enabled us to localize the sensitive sulphhydryl group(s) to either of these fragments. Soluble fragments were prepared as previously described³, by subjecting EDTA-stripped and KCl-extracted microsomal vesicles to low concentrations of trypsin (7 $\mu\text{g ml}^{-1}$). Although such a mild treatment does not completely separate the membrane from the soluble portion of the translocation apparatus, it avoids the rapid inactivation of the solubilized fragments observed at higher concentrations of trypsin³. Centrifugation yielded a supernatant fraction that was further incubated in the absence or presence of *N*-ethylmaleimide. These fractions are referred to as *t*₇-sup or *t*₇-n-sup, respectively.

Membrane-associated fragments, on the other hand, were prepared by digestion of EDTA-stripped, salt-extracted microsomal vesicles with a high concentration of trypsin (60 $\mu\text{g ml}^{-1}$). This harsher treatment generates a membrane fraction that is completely translocation inactive by itself (see Figs 1, 2, lane 7)

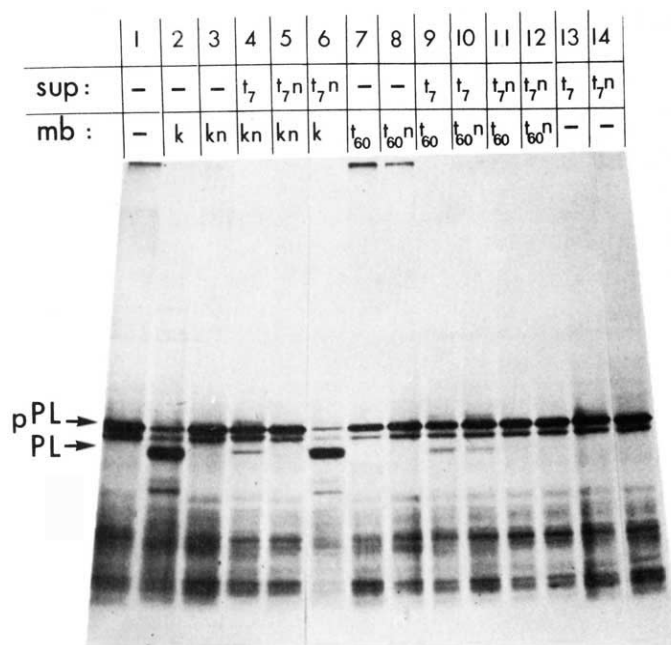


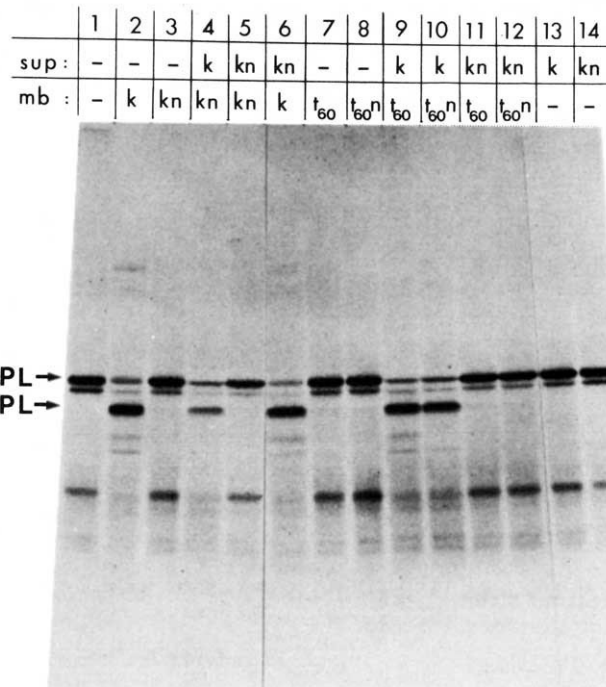
Fig. 1 The sulphhydryl group required for translocation is located on a cytoplasmically disposed, trypsin-sensitive domain of the translocation apparatus. Supernatant (sup) and membrane (mb) fractions were prepared from mildly (7 $\mu\text{g ml}^{-1}$) and extensively (60 $\mu\text{g ml}^{-1}$) trypsin-digested samples of EDTA and KCl-stripped rough microsomes (k-mb) as previously described³. Aliquots of these fractions were reacted with 1.5 mM *N*-ethylmaleimide for 30 min at 25 °C, then quenched with a 10 fold molar excess of dithiothreitol (DTT). Both modified and unmodified fractions were assayed for their ability to support and/or reconstitute the translocation of preprolactin across the microsomal membrane. Samples of the indicated fractions were added to a wheat-germ *in vitro* translation system¹⁰ programmed with bovine pituitary RNA and supplemented with human placental ribonuclease inhibitor¹¹ at a final concentration of 0.006 A₂₈₀ units ml⁻¹. In assaying for the reconstitution of translocation by the *t*-sup fractions, 3 μl of the indicated supernatant fraction were added to a typical 25- μl translation mixture. (3 μl of *t*-sup are derived from 0.126 A₂₈₀ units of stripped microsomes.) Where indicated, membranes were present at a final concentration of 2.3 A₂₈₀ units ml⁻¹. Translation products were separated by polyacrylamide gel electrophoresis in SDS, and detected by autoradiography. Translocation capacity is assessed from the ability of the added fractions to process preprolactin (pPL) to prolactin (PL). The membrane fractions are: k-mb, KCl and EDTA-stripped rough microsomes; kn-mb, *N*-ethylmaleimide-modified k-mb; t₆₀-mb, extensively (60 $\mu\text{g ml}^{-1}$) trypsinized k-mb; t₆₀n-mb, *N*-ethylmaleimide-modified t₆₀-mb. The supernatant fractions are: t₇-sup, supernatant from mildly (7 $\mu\text{g ml}^{-1}$) trypsinized k-mb; t₇-n-sup, *N*-ethylmaleimide-modified t₇-sup.

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Fig. 2 A factor removed from EDTA-stripped rough microsomes by extraction with 0.5 M KCl also contains an essential sulphhydryl moiety. EDTA-stripped rough microsomes were extracted with 0.5 M KCl, as described by Warren and Dobberstein². Aliquots of the KCl extract (k-sup) and membrane (k-mb) fractions were modified with 1.5 mM *N*-ethylmaleimide as described in Fig. 1 legend. Samples of the modified and unmodified supernatant and membrane fractions were assessed for their ability to support and/or reconstitute translocation activity as indicated by their ability to process preprolactin to prolactin. In assaying for the reconstitution of translocation by the t-sup and k-sup fractions, 3 μ l of these supernatant fractions were added to a typical 25- μ l translation mixture. (3 μ l of k-sup are derived from 0.154 A_{280} units of EDTA-stripped microsomes.) Where indicated, membranes were present at a final concentration of 2.3 A_{280} units ml^{-1} . The abbreviations used are: k-sup, 0.5 M KCl extract of EDTA-stripped rough microsomes; kn-sup, *N*-ethylmaleimide-modified k-sup; other abbreviations are as in Fig. 1.

but retains its competence to serve as acceptor for the solubilized fragment(s) and thereby to restore translocation activity (Fig. 1, lane 9). Before assaying for translocation activity, the trypsinized membrane fraction was further incubated in the absence or presence of *N*-ethylmaleimide. The resulting fractions are referred to as t_{60} -mb or t_{60n} -mb, respectively.

The effects of *N*-ethylmaleimide treatment of these various fractions as well as some essential controls are shown in Fig. 1. Two conclusions can be drawn from the data. First, *N*-ethylmaleimide inactivated only the trypsin-solubilized fragments, but not the membrane-integrated remainder of the translocation apparatus (compare lanes 11, 12 with 9, 10). Thus, the important sulphhydryl group(s) is located in the cytosol-exposed, trypsin-sensitive domain of the translocation apparatus. Furthermore, as *N*-ethylmaleimide readily permeates membranes, and as reconstitution of translocation is not affected by *N*-ethylmaleimide modification of t_{60} -mb (Fig. 1, lane 10), the residual t_{60} -mb cannot contain any sulphhydryl groups essential for translocation, that is, the essential sulphhydryl is located exclusively on the cytosol-exposed domain of the translocation apparatus. Second, non-trypsinized control membranes (k-mb) must contain membrane-integrated fragments comparable to



those that are experimentally generated by trypsinization (t_{60} -mb fraction). This was previously suggested by the finding that the translocation activity of trypsin-inactivated membranes could be increased to levels greater than those of untrypsinized membranes by the addition of saturating concentrations of the trypsin-solubilized supernatant factor³. Here it is indicated by the finding that a non-trypsinized, but *N*-ethylmaleimide-modified membrane fraction (kn-mb) which is translocation inactive (see Fig. 1, lane 3) can be partially reactivated by a t_{60} -sup fraction (lane 4). In other words, the control,

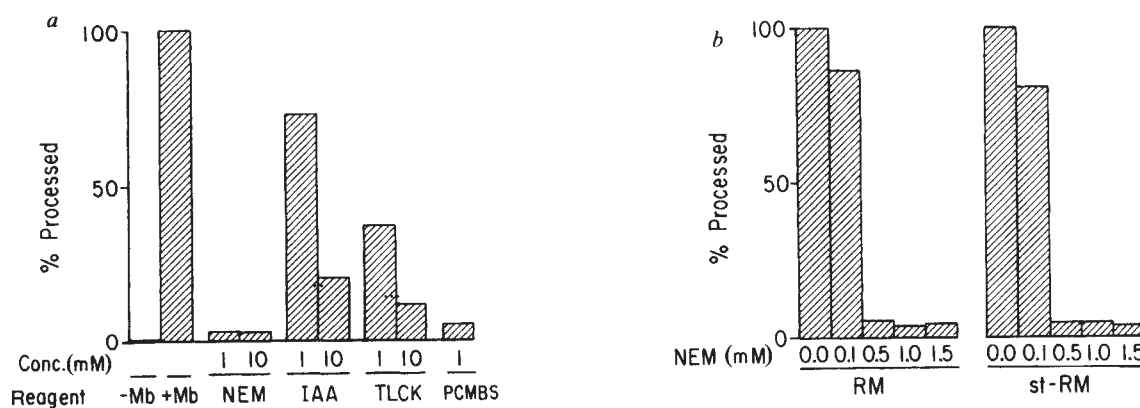


Fig. 3 *a*, Inhibition of precursor processing (and translocation) by sulphhydryl-modifying reagents. Stripped rough microsomes (st-RM) were prepared as previously described^{3,12} with the exception that DTT was excluded from all buffers. Modification reactions (50 μ l) contained stripped microsomes at a final concentration of 46.5 A_{280} units (determined in 5% SDS), 1% Trasylol, 0.2 M sucrose, 100 mM triethanolamine-HCl (TEA-HCl), pH 7.5, 40 mM KCl, 0.8 mM $MgCl_2$ and the indicated concentration of freshly prepared sulphhydryl-modifying reagent. Reactions were quenched after 30 min at 25 °C by the addition of at least a 10-fold molar excess of DTT. The DTT-inactivated sulphhydryl-modifying reagents were removed from the modified stripped microsomes by centrifugation in a Beckman cellulose nitrate air-liquid interface tube containing a sucrose step gradient comprised of 80 μ l of 0.5 M sucrose, 10 mM TEA-HCl, pH 7.5, 1 mM DTT and 25 μ l of 2.0 M sucrose in the same buffer. After centrifugation (5 min, at 30 lb per sq. inch, 100,000 g_{av}) the microsomes were removed from the 2.0–0.5 M sucrose interface with a Hamilton syringe, brought to a total volume of 20 μ l with 10 mM TEA-HCl, pH 7.5, and 1 mM DTT, and assayed for their ability to support translocation (see text) and processing of preprolactin to prolactin. Translocation assays were carried out in a final volume of 50 μ l in a wheat-germ translation system¹⁰ supplemented with human placental ribonuclease inhibitor¹¹ at a final concentration of 0.006 A_{280} units ml^{-1} . The wheat-germ system was programmed with pituitary RNA and supplemented with the appropriately modified membranes at a final concentration of 2.3 A_{280} units ml^{-1} . The ³⁵S-Met-labelled products were separated by gel electrophoresis in SDS, detected by autoradiography of the dried gel and quantitated by excising and counting the portion of the gel containing the ³⁵S-Met-labelled preprolactin and prolactin products^{3,12}. Processing is expressed as per cent of control, unmodified membranes (+mb). In this particular experiment, the absolute amount of preprolactin processed by the unmodified membranes was 35.0% of the total preprolactin synthesized. The abbreviations used are: *N*-ethylmaleimide, NEM; iodoacetamide, IAA; *p*-chloromercuribenzenesulphonic acid, PCMBs; *N*- α -*p*-tosyl-L-lysine chloromethyl ketone, TLCK; sodium dodecyl sulphate, SDS. *b*, The processing (and translocation) activity of both rough and stripped rough microsomes is inhibited by *N*-ethylmaleimide modification. A rough microsomal fraction (RM), 107 A_{280} units ml^{-1} , was divided into two samples. Ribosomes were removed from the microsomes of one of these samples by mixing the sample with one volume of a ribosome-stripping solution containing 0.25 M sucrose, 40 mM EDTA and 50 mM TEA-HCl, pH 7.5. The stripped microsomes (st-RM) were separated from EDTA by centrifugation. Aliquots of both the stripped and the unstripped microsomes were modified with the indicated concentration of *N*-ethylmaleimide (NEM) as described in *a*. After inactivation of residual *N*-ethylmaleimide with DTT, ribosomes were removed from the rough microsomal fractions by EDTA extraction, as described above. The modified, stripped microsomes were separated from inactivated *N*-ethylmaleimide and EDTA by centrifugation and assayed for translocation activity (see *a*). In this particular experiment, the absolute amount of preprolactin processed by the unmodified stripped microsomes was 74.5% of the total preprolactin synthesized.

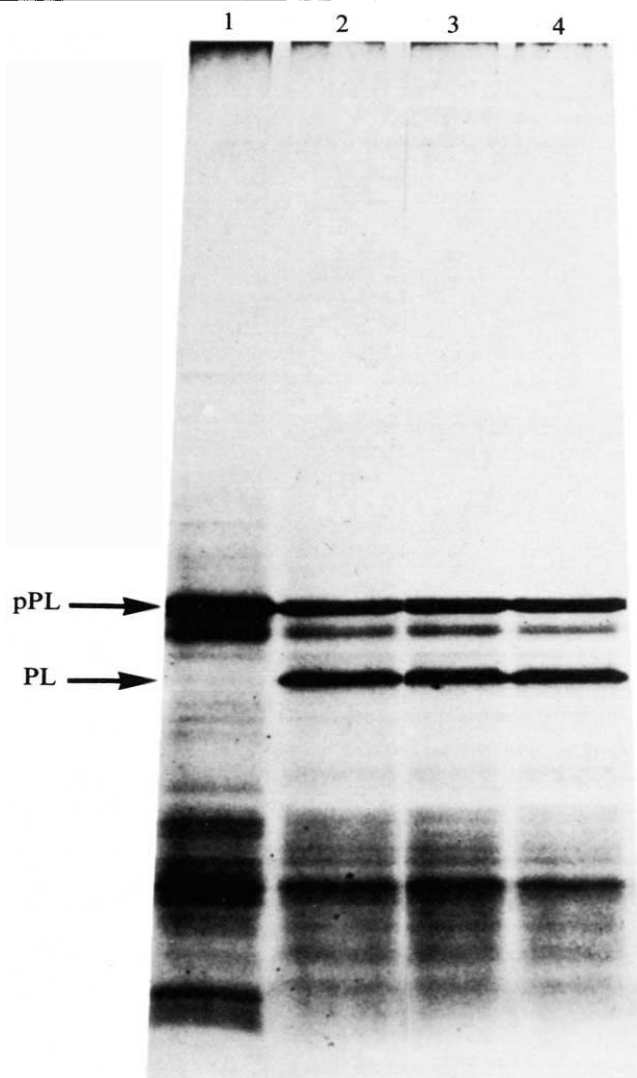


Fig. 4 Aliquots (40 μ l) of stripped microsomes (56 A_{280} units ml^{-1}) were modified with the indicated concentration of *N*-ethylmaleimide as described in Fig. 3a legend. After termination of the modification reaction with DTT (see Fig. 3a), the microsomes were dissociated by the addition of sodium deoxycholate to a final concentration of 0.5% (w/v) and were assayed for the ability of signal peptidase to process full-length ^{35}S -Met-preprolactin (pPL) as previously described⁸. Lane 1: control, unprocessed preprolactin; lane 2: processing by unmodified, deoxycholate-dissociated microsomes; lanes 3, 4: processing by microsomes which had been modified with 10 mM (lane 3) or 20 mM (lane 4) *N*-ethylmaleimide before deoxycholate dissociation.

untrypsinized membranes behave as if they had already been subjected to limited proteolytic degradation, resulting in the production of some nonfunctional but reactivatable translocation sites. As expected, the observed reactivation of the kn-mb fraction is eliminated when a t_7 -sup fraction (Fig. 1, lane 5) is substituted for the t_7 -sup fraction.

In the controls in Fig. 1, we show that neither the t_7 -sup (lane 13) nor the t_7 -n-sup (lane 14) fractions are themselves capable of supporting translocation in the absence of added membrane. An additional control (lane 6) demonstrates that the t_7 -sup fraction does not inactivate translocation by added k-mb, indicating that our procedures (see Fig. 3a) for quenching *N*-ethylmaleimide are effective and that the observed effects of the *N*-ethylmaleimide-modified fractions are not due to a carryover of residual *N*-ethylmaleimide.

The presence of nonfunctional, but reactivatable translocation sites in untrypsinized k-mb suggested that the 'salt factor' of Warren and Dobberstein² is in reality a salt-extractable, proteolytic fragment of the translocation apparatus, comparable to that experimentally generated by trypsin. Therefore, one would expect that the salt factor, like the trypsin-generated soluble fragment(s), might also contain a sulphhydryl

group(s) that is essential for activity. These expectations were indeed borne out by data shown in Fig. 2. Although in our hands KCl extraction does not result in a complete loss of translocation activity, our KCl extract (k-sup) fraction does contain a salt factor which is able to restore translocation activity to trypsin-inactivated microsomes (Fig. 2, lane 9), to *N*-ethylmaleimide inactivated microsomes (Fig. 2, lane 10) and to microsomes inactivated by both trypsin and *N*-ethylmaleimide (Fig. 2, lane 4). In fact, the ability of the KCl extract to restore translocation is quantitatively superior to that of the tryptic extract (compare Figs 1 and 2, lanes 4, 9, 10). Although this difference in activity may reflect a genuine difference in the absolute reactivation capacity of the KCl and tryptic factors, other explanations (particularly tryptic inactivation of a substantial portion of the trypsin-solubilized factor) are also likely. Nevertheless, like its trypsin-generated counterpart, the salt factor is readily inactivated by *N*-ethylmaleimide (Fig. 2, compare lanes 5, 11, 12 with lanes 4, 9, 10). The control (Fig. 2, lane 6) demonstrates that the *N*-ethylmaleimide-inactivated salt factor does not affect translocation of EDTA-stripped, KCl-extracted membranes (Fig. 2, compare lane 6 with lane 3), indicating that the observed effects of the *N*-ethylmaleimide-modified fractions are not due to a carryover of residual *N*-ethylmaleimide.

From these experiments, we conclude that the translocation-reactivating factors present in k-sup and t_7 -sup are functionally and structurally similar, in that both restore translocation activity to *N*-ethylmaleimide- or trypsin-inactivated test membranes, and both contain an essential sulphhydryl group(s). Although both these factors are probably derived from the same parent molecule, differences in their extractability (the trypsin factor is released in the absence of salt) suggest that tryptic digestion removes a smaller fragment of the parent translocator protein than is generated by the endogenous tissue protease(s) which produces the salt factor. Presumably, it is this additional domain of the salt factor which is responsible for the differential salt extractability of the salt and tryptic factors. In any case, the observation that the cytoplasmically disposed domain of the translocation apparatus contains an essential sulphhydryl which can be readily modified provides us with a tool which should be extremely useful in the purification and characterization of the polypeptides responsible for translocation of nascent secretory and membrane proteins across the membrane of the rough endoplasmic reticulum.

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Efficiency of the adaptive response of *Escherichia coli* to alkylating agents

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When cultures of *Escherichia coli* are exposed to a low level of the alkylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) they accumulate mutations for about 20 min and then

become resistant to further mutagenesis by that level of MNNG¹. This 'adaptive response,'² has been shown to be due, at least in part, to induction of the rapid repair of *O*⁶-alkyl-guanine³⁻⁵ which appears to be the main mutagenic and carcinogenic lesion produced by simple alkylating agents^{6,7}. A similar kind of repair has been demonstrated in the livers of rats exposed to nitrosamines⁸, and this presumably helps to protect animals against carcinogenesis by the various alkylating agents that are widespread in our environment. It seemed important, therefore, to find out just how effectively such adaptive responses can control mutation rates.

The very efficiency of the response of *E. coli* to MNNG imposed certain constraints on the experiment to measure residual mutation rates. To prepare cultures of adapted bacteria which are free from the mutants that initially arose when adaptation was being induced, it is necessary to start each culture with only a few thousand adapted bacteria. But measurement of the subsequent very low mutation rate of bacteria growing in the presence of the level of MNNG to which they are adapted requires samples of at least 10^7 bacteria. It was therefore necessary to prepare a low pH, low phosphate⁹ growth medium in which MNNG was sufficiently stable to survive for more than 12 bacterial generations (10–15 h). In practice, the stability of MNNG in such a medium is best checked by observing the distribution of mutant clone sizes in replicate cultures; for, if its concentration is effectively unchanged for the course of the experiment and the mutation rate (m) per cell per generation is therefore constant, the proportion of cultures of N bacteria that contain x or more mutants will follow the distribution of clone sizes, described by Luria¹⁰ for mutations that arise at random in an exponentially increasing population: namely

$$x \sum_{i=x}^{i=N} P_i \rightarrow 2mN, \quad \text{as} \quad \frac{2mN}{x} \rightarrow 0 \quad (1)$$

and the proportion of cultures that contain one or more mutants will be

$$\sum_{i=1}^{i=N} P_i = 1 - \exp(-2mN) \quad (2)$$

[In the present instance, it is not necessary to consider the time of duplication of different genes in the cell cycle, because the experiments are solely concerned with comparisons between mutation rates at a single locus and furthermore do not deal with fractional generation times.]

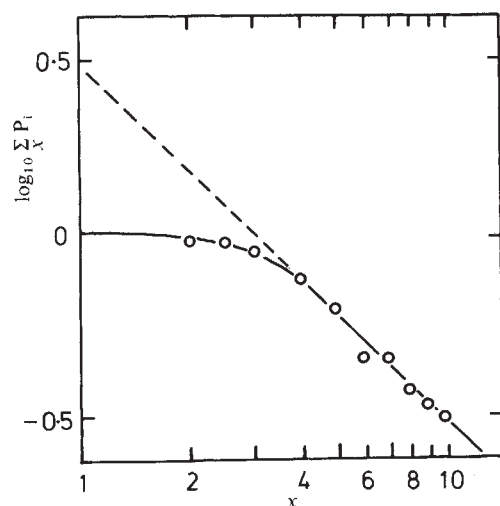


Fig. 1 The distribution of arg^+ revertant clone sizes in cultures of *E. coli* AB 1157 growing in the presence of $0.5 \mu\text{g ml}^{-1}$ MNNG. If the mutation rate is constant, the proportion of such cultures that contain x or more revertants will approach $2mN/x$ (equation (1)), and the best estimate of mN can be obtained by fitting a line of slope -1 to a plot of the logarithm of these proportions against $\log x$ and then measuring the intercept at $x = 1$. Here, the intercept gives $\log(2mN)$ equal to 0.48 , or $2mN$ equal to 3 . As N equalled 4×10^7 , m equals 4×10^{-8} .

From either of these equations it is possible to measure the spontaneous mutation rate of unadapted bacteria growing in the absence of MNNG and the overall mutation rate (provoked plus spontaneous) of adapted bacteria growing in the presence of the concentration of MNNG to which they have been adapted. What the MNNG-provoked mutation rate would have been, in the absence of the adaptive response, can be determined simply by exposing a strain that cannot induce the response¹¹ to the medium in which the adapted bacteria had been growing; after a period of one generation these non-adapting bacteria will contain mN mutants.

The results of such an experiment are shown in Table 1 and Fig. 1. Most mutant clones in the adapted cultures are small (that is, of recent origin) and have the expected distribution (Fig. 1). The extra mutation rate in the MNNG-adapted cultures was $3.5 \times 10^{-8} \text{ arg}^+$ per generation, but the medium in which these bacteria were growing produced $20,000 \times 10^{-8} \text{ arg}^+$ per generation in an adaptation-deficient strain. In these particular conditions, therefore, the adaptive response was lowering the mutation rate about 6,000-fold. This is a surprising result. Each DNA base in *E. coli* serves as template once every 2,500 s. For adaptation to lower 6,000-fold the probability that an altered base serves as a source of mutation, the adaptive response may have to render each premutational lesion such as *O*⁶-methylguanine harmless within 0.5 s: (of course, the process would not have to be completed so quickly if the enzymes for post-replication mismatch repair can recognize and correct structures such as *O*⁶-methylguanine-thymine base pairs). Conceivably, the reaction succeeds in being rapid because the catalytic reactant restores the normal base-pairing properties of the guanine

Table 1 Measurement of the mutation rate produced by $0.5 \mu\text{g ml}^{-1}$ MNNG in bacteria that are either adapted or unable to adapt

	Cells plated	<i>arg</i> ⁺ revertants	Mutation rate per generation × 10 ⁸
AB 1157	4 × 10 ⁷	0, 1, 1, 1, 1, 1, 1, 1, 1, 2, 2, 5	0.5*
AB 1157 in 0.5 µg ml ⁻¹ MNNG	4 × 10 ⁷	0, 2, 2, 3, 3, 3, 3, 3, 4, 4, 4, 4, 5, 5, 5, 5, 5, 7, 7, 7, 8, 9, 13, 19, 23, 42, 55, 89, 239, ~2,000	4.0†
AB 1157 <i>ada</i> 5	6 × 10 ⁷	0, 0, 0, 0, 1, 1, 1, 1	~0.4*
AB 1157 <i>ada</i> 5 1 hr in supernates of MNNG cultures	1 × 10 ⁶	174, 181, 192, 196, 204, 204, 211, 231	20,000

A culture of *E. coli* AB 1157 (*arg*, *his*, *leu*, *pro*, *thr*, *thi*) was grown in a low pH, low phosphate medium containing 0.1 M NaCl, 0.04 M KCl, 0.02 M NH_4Cl , 1 mM MgCl_2 , 0.5 mM KH_2PO_4 , 0.2 mM Na_2SO_4 , 0.1 mM CaCl_2 , 0.01 mM FeCl_3 , 0.025 M Tricine (Sigma) pH 6.1, plus required amino acids ($20 \mu\text{g ml}^{-1}$) and vitamins ($0.1 \mu\text{g ml}^{-1}$) and 0.6% glucose. When the bacterial concentration had reached 10^7 per ml, MNNG was added to give a level of $0.1 \mu\text{g ml}^{-1}$ for one generation and $0.5 \mu\text{g ml}^{-1}$ for a further generation. At this point the culture was diluted to 2,000 bacteria per ml, and 32 3-ml aliquots were prepared in the absence of MNNG and 32 in the presence of $0.5 \mu\text{g ml}^{-1}$ MNNG. After 18 h incubation (when the cultures contained, by microscope count, 2×10^7 bacteria per ml), each aliquot was centrifuged, resuspended in 0.5 ml minimal medium and plated on minimal plates lacking arginine, to give the number of *arg*⁺ revertants. These two sets of cultures therefore provided estimates of the spontaneous reversion rate and the sum of spontaneous and MNNG-provoked mutation rate to *arg*⁺. At the same time, the supernatant medium from the MNNG-containing cultures was used to suspend similar cultures of the adaptation-defective strain AB 1157 *ada* 5 (ref. 11). After exposure for 1 h (roughly one generation) these cultures were centrifuged and similarly plated on arginine-deficient plates.

* Mutation rate determined by equation (2).

† Mutation rate determined by equation (1) (see Fig. 1).

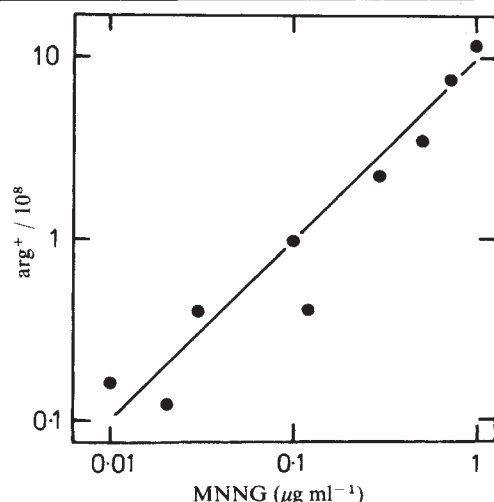


Fig. 2 The extra mutation rate of *E. coli* AB 1157 growing in the presence of various concentrations of MNNG. The mutation rate (*arg*⁺ revertants per generation) was determined as described in Table 1 and Fig. 1; the spontaneous rate (1.2×10^{-9}) has been subtracted.

in one step by providing an alternate site of attachment for the alkyl group⁵, and this is why each reactant molecule apparently can act only once⁴. Whatever the mechanism, it is interesting that the adaptive response succeeds in being at least as efficient as the 'error-free' repair of pyrimidine dimers produced by ultraviolet light^{12,13} even though dimers are presumably much less of a threat because, unlike alkylated bases, they cannot be copied and so are not an immediate source of mutation.

The above calculation was based on the assumption that the low residual mutation rate in adapted bacteria is due to the very rare occasions when an *O*⁶-methylguanine happens to be copied before it is repaired; and if that is correct, the mutation rate in adapted cultures should be directly proportional to the rate of production of alkylated bases (that is, proportional to mutagen concentration). However, more complex relationships between dose and response are imaginable. For example, the adaptive response could be 100% efficient once induced, but a small proportion of the cells might fail to undergo induction; in this case, very low levels of mutagen might be more mutagenic than higher levels. Such questions are of practical importance in assessing the likely risks from low levels of carcinogens, and so these experiments with bacteria were extended to cover a range of MNNG concentrations.

As Fig. 2 shows, adapted cultures of *E. coli* growing in the presence of MNNG have an increase in mutation rate that is approximately proportional to MNNG concentration. Interestingly, a similar result has been seen when rats are continuously exposed to dimethyl or diethyl nitrosamine in their drinking water (R. Peto, personal communication). Doses below 1 p.p.m. (that is, below about 0.1 mg per kg per day) produced an incidence of liver cancer that was roughly proportional to dose; but between 1 and 10 p.p.m. the age-specific incidence increased about 1,000-fold. For these alkylating agents it seems clear, therefore, that brief exposures are much more mutagenic for certain bacteria and high dose rates are much more carcinogenic for certain animals than might be expected from the response to continuous low dose rates.

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Virus-specific effects of interferon in embryonal carcinoma cells

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Embryonal carcinoma (EC) cells are susceptible to infection by a variety of viruses¹, but do not become resistant to infection by Semliki Forest virus² or vesicular stomatitis virus³ (VSV) on treatment with interferon. These observations have led to the conclusion that interferon does not induce an antiviral state in EC cells^{2,3}. We report here, however, that EC cells treated with interferon become resistant to infection by two picornaviruses and two *ts* mutants of VSV, whereas they remain sensitive to wild-type VSV, Sindbis and influenza virus infection. These results suggest that a partial antiviral state is induced in EC cells by interferon and that the induced antiviral protein(s) interferes with the replication of specific viruses. A significant common feature of these viruses is their replication through structures containing double-stranded RNA (dsRNA)⁴.

Interferon induces in human and mouse cells the synthesis of a few proteins that are detected by gel electrophoresis^{5,6}. Among these are two enzymes, an oligonucleotide polymerase and a protein kinase (see ref. 7 for refs), which are activated by dsRNA in extracts of interferon-treated cells. The polymerase converts ATP into a series of oligonucleotides characterized by 2'/5'-phosphodiester bonds and designated 2-5A (ref. 8). The 2-5A activates an endoribonuclease that degrades RNA^{9,10}. The protein kinase (PK_{ds}) inhibits protein synthesis by phosphorylating the α subunit of initiation factor eIF-2 (ref. 7). Wood and Hovanessian³ assayed these enzymatic activities in extracts of interferon-treated EC cells and observed an increase in 2-5A polymerase activity comparable to that in mouse L cells, whereas the PK_{ds} was not significantly increased in EC cells. As these cells did not become resistant to VSV infection³, the induction of 2-5A polymerase could not be correlated with an antiviral state. In a different cell system, however, the induction of 2-5A polymerase is closely correlated with the inhibition of replication of a picornavirus, encephalomyocarditis virus

Table 1 Effect of interferon on virus yield and induction of 2'/5'-oligo(A) polymerase and protein kinase

Cells	PCC4		L	
Interferon treatment	-	+	-	+
EMCV yield	8	3	8	4
VSV yield	8	8	8	4
2'/5'-oligo(A) synthesis	10	680	10	2,440
Protein kinase	2	2	2	30

Cultures of control and interferon-treated cells ($100 \text{ units ml}^{-1}$ for 20 h) were infected for 48 h as described in Fig. 1 legend. The cultures were frozen and thawed three times and centrifuged for 10 min at $30,000g$. The virus yield was measured by end-point dilution of the supernatant and quantification of the cytopathic effect on L-cell monolayers. Virus samples of known titre were assayed in parallel; virus yield is expressed as the log₁₀ of the titre in plaque-forming units per ml. Cytoplasmic extracts were prepared from control and interferon-treated cells grown in 75-cm² flasks. The cells were washed with saline and homogenized as described elsewhere¹⁶, with the addition of 0.5% Triton X-100 to the homogenization buffer. The S30 fraction obtained by centrifugation for 5 min at $30,000g$ was assayed for 2-5A synthesis with poly(I)·poly(C)-paper as described by Stark *et al.*²⁶. The synthesis of 2-5A obtained with $100 \mu\text{l}$ of S30 in 16 h is expressed in pmol of ATP converted into oligonucleotides out of an input of 250 nmol of ATP per 50- μl reaction. The protein kinase activity was assayed by measuring the phosphorylation of a 67,000 molecular weight polypeptide as described elsewhere²⁷. The S30 was centrifuged for 90 min at $150,000g$ and the pellet obtained resuspended and incubated with [³²P]ATP and $1 \mu\text{g ml}^{-1}$ of poly(I)·poly(C); the phosphorylated proteins were resolved by gel electrophoresis, detected by autoradiography and quantified by scanning the autoradiographs as described elsewhere²⁷. The protein kinase activity is expressed in units of peak area, relative to a reference polypeptide present in both L and PCC4 cells.

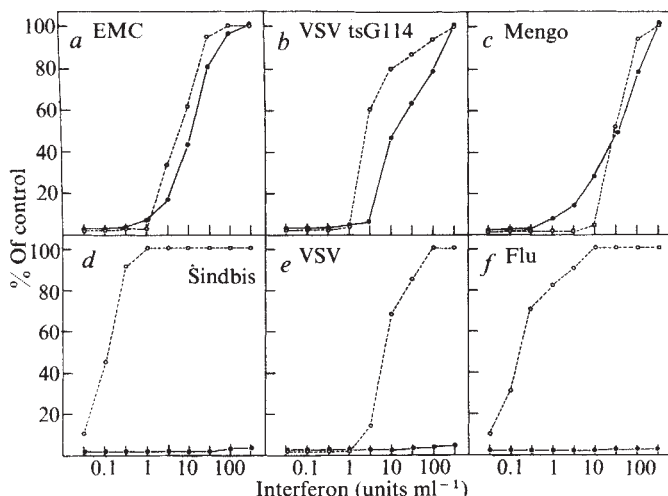


Fig. 1 Antiviral effects of interferon in embryonic carcinoma (●—●) and L cells (○—○) infected with different viruses. The PCC4AzaR1 and L929 cell lines were maintained in monolayer cultures in Dulbecco modified Eagle's medium containing 10% heat-inactivated calf serum. Before infection the cells were seeded at about 3×10^5 cells per 16-mm well in 24-well cluster plates (Costar). The amount of virus required to produce a complete cytopathic effect in 48 h was determined for both cell types by end-point dilution of concentrated virus stocks. The cytopathic effect was quantified by the viral dye uptake method¹⁵. The L cells were infected by adding 0.2 ml of virus stock diluted in medium minus serum to each well. After 1 h at 37 °C for all viruses except the *ts* mutants of VSV, which were kept at 30 °C, 0.8 ml of medium containing 1% fetal calf serum was added and the infection continued for 48 h. As the PCC4 cells were infected more effectively while the cells were not adhering to the plastic wells, these cells were resuspended by shaking, infected and allowed to re-attach. Treatment with the concentration of interferon indicated in the abscissa (NIH reference units) was for 20 h before infection. At the start of the interferon treatment the PCC4 cells were suspended as described above for virus infection. The cytopathic effect was quantified after 48 h of infection. In all experiments control infected and non-infected cells were examined in parallel cultures. The A_{540} of the dye taken up by the latter cells was taken as 100%; the A_{540} of the control infected cells represents the 0% value shown in the ordinate. Typical A_{540} values were 2.5 for uninfected cells and 0.15 for untreated infected cells. Mouse interferon was obtained from Ehrlich ascites tumour cells infected with Newcastle disease virus and partially purified to about 10^7 units per mg of protein¹³. The viruses used are indicated. Vesicular stomatitis virus (VSV) was the Indiana serotype strain. Identical results were obtained with both VSV *ts* mutants G114 and G11 and therefore the results with tsG114 only are shown. Flu designates the WSN strain of influenza virus.

(EMCV)¹¹. Moreover, this inhibition is observed even when induction of PK_{ds} is prevented by actinomycin D (ref. 11). In view of these observations, it was of interest to investigate the response of interferon-treated EC cells to picornavirus infection.

The EC cell line PCC4, derived from the transplantable ascitic form of the OTT 50-60 teratocarcinoma¹², and L929 cells were grown in monolayer cultures. These cells were treated with increasing concentrations of mouse interferon prepared from Ehrlich ascites tumour cells¹³ and infected with different viruses as described in Fig. 1 legend. The viruses were: EMCV and Mengo, two picornaviruses; VSV and its two mutants tsG11 and tsG114 (ref. 14); Sindbis and influenza virus. All the viruses infected both L and EC cells, as shown by quantification of the cytopathic effect by a viral dye uptake method¹⁵. Low concentrations of interferon protected L cells from all viruses tested. In contrast, concentrations up to 300 units ml⁻¹ of interferon did not protect EC cells from VSV, Sindbis or influenza virus. Interferon concentrations comparable to those effective for L cells, however, protected EC cells from infection by EMCV, Mengo and the VSV mutants.

To confirm that interferon has a protective effect on EC cells, we also measured virus yield in control and interferon-treated cells infected with EMCV and VSV (Table 1). Treatment with interferon reduced virus yield by four logs in both cells infected with EMCV, but had no effect on the yield of VSV in EC cells. The protective effect of interferon in EC cells, therefore, is clearly virus specific.

In subsequent experiments, we confirmed that only one of the two enzymatic activities induced by interferon is elevated in EC cells, whereas both are increased in L cells (Table 1). The 2-5A polymerase was induced in EC cells by interferon, but the kinase was not. The 2-5A synthesized by EC and L cell extracts was characterized by its elution from DEAE-cellulose with 0.35 M KCl¹⁶ (Table 1) and by its biological activity in an endonuclease activation assay⁹. Oligonucleotides synthesized with extract of interferon-treated PCC4 and L cells activated a HeLa cell endonuclease, whereas corresponding fractions from incubations with extract of control cells did not (data not shown).

These observations suggest a possible correlation between the inhibition of replication of specific viruses in EC cells and the induction of the 'antiviral protein' 2-5A polymerase. Although the precise mechanism of action of this enzyme has not been determined, the following observations suggest that the 2-5A polymerase has some role in preventing replication of picornaviruses: (1) 2-5A is formed in interferon-treated cells infected with EMCV¹⁷; (2) 2-5A introduced into intact cells inhibits protein synthesis and causes RNA breakdown^{18,19}; (3) preferential degradation of viral RNA is observed when the replicative intermediate (RI) of EMCV is incubated with extracts of interferon-treated cells (the RI is the RNA component of the replicative complex)²⁰; (4) EMCV replicates by forming base-paired structures. This was shown by isolating cross-linked RI from infected cells treated with aminomethyl-trioxalen⁴. This compound intercalates into duplexes and forms covalent bonds on irradiation²¹. Cross-linked RNA could not be detected in cells infected with wild-type VSV, but was detected in cells infected with two *ts* mutants that are sensitive to interferon protection in EC cells⁴.

Interferons are glycoproteins that are operationally defined in terms of their antiviral activity against a wide range of viruses. With the recent advances in our understanding of the molecular basis of the antiviral state⁷, it has become clear that several defence mechanisms are activated in interferon-treated cells; for example, an inhibitor of viral mRNA methylation²² and a reduction in the amount of G protein incorporated into VSV²³ have been reported. The fact that interferon does not protect EC cells from VSV infection presumably means that these latter defence mechanisms are not operational in EC cells. In most cells interferon activates a spectrum of antiviral defence mechanisms, whereas in EC cells it elicits a restricted antiviral state, possibly limited to the induction of 2-5A polymerase.

A better understanding of the different antiviral mechanisms and of the replication of different viruses may ultimately provide a satisfactory explanation for the present and previous observations. A genetically determined virus-specific effect of interferon, for example, is due to the *Mx* allele in A2G mice²⁴. Haller *et al.*²⁵ have reported that macrophages of *Mx* mice can be protected from influenza virus by much smaller interferon concentrations than are necessary to protect macrophages of other mice; no difference in protection is observed, however, on infection with EMCV or VSV. These authors speculate that the antiviral state is composed of a multitude of specific antiviral mechanisms and envisage the *Mx* gene as regulating the expression of a component of the antiviral state²⁵. Each component may be both genetically and developmentally regulated, as shown by this report and by our observations on embryonic carcinoma cells.

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A new papillomavirus associated with alimentary cancer in cattle

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A high incidence area of carcinoma of the alimentary tract of cattle has been reported^{1–4}. The carcinomas are preceded by papillomas which may transform to malignancy. A papillomavirus was isolated from these warts and was shown to occur as a widespread infection of cattle in the UK². In the high cancer area there is a marked increase in the overall incidence of this infection, an increase in the number of papillomas per animal and an increase in the number of specific sites of infection as compared with cattle from adjacent areas where cancers are not found. The papillomas are found in young animals but carcinomas do not develop until the cows are over 7 yr old. There is strong circumstantial evidence¹ that the factor associated with the increased multiplicity of papillomas and the subsequent malignant transformation is the ingestion of bracken fern. This plant is known to possess radiomimetic, mutagenic and carcinogenic activity. Preliminary experimental evidence supports the circumstantial data and we also have preliminary evidence of the presence of viral genome sequences, without production of virus, in the cells of carcinomas. In addition to the alimentary papillomas of the type described above, three more major patterns of bovine papillomatosis are found in the UK⁵—cutaneous fibropapillomatosis, papillomatosis of the skin of the udder and teats, and papilloma of the penis. We have extracted papillomaviruses from all the above tumours, and our results, published in a preliminary form elsewhere⁵, indicate that the different lesions are caused by different papillomaviruses. Here we report the partial characterization of the virus involved in alimentary tract tumours and its differentiation from other bovine papillomaviruses. The system seems to present a dissectable model for investigating the mechanistic relationship between the viral infection and the ingestion of an environmental 'carcinogen'.

Virus was isolated from a variety of papillomas by homogenization and density gradient centrifugation on CsCl. The papillomas were of four of the types recognized in cattle in the UK⁵. Alimentary papillomavirus (BAPV) was obtained from oesophageal warts; each isolate was made from a single abattoir animal which had several tumours but no concurrent carcinoma, and no other type of papillomas. DNA was purified from the virus and was present in two forms, twisted circles (form 1) and relaxed circles (form 2) (Fig. 1*b*). In agarose gel electrophoresis, both forms migrated ahead of the corresponding forms of DNA isolated from the virus (BCPV) of cutaneous fibropapillomas

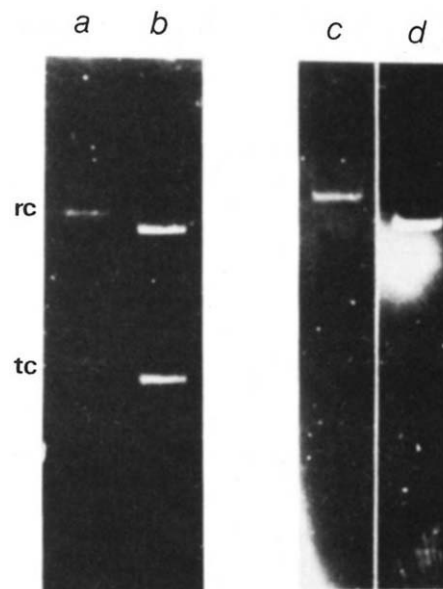


Fig. 1 Comparison between BCPV and BAPV DNA. *a*, Unrestricted BCPV DNA, and *b*, unrestricted BAPV DNA, electrophoresed in a 1% agarose gel. rc, relaxed circles; tc, twisted circles. *c*, *Hind*III-restricted BCPV DNA, and *d*, *Bam*HI-restricted BAPV DNA, electrophoresed in a 0.7% agarose gel. The MWs ($\times 10^{-6}$) are shown on the right. The MW of BAPV DNA was calculated by using the known MW of BCPV DNA as reference. Cutaneous and oesophageal papillomas were finely homogenized in TS buffer (10 mM NaCl, 1 mM Tris pH 8.0, 1 mM EDTA). The debris was pelleted at 2,000 r.p.m. for 5 min and the supernatant centrifuged at 10,000g for 15 min. Virions were pelleted from the 10,000g supernatant by centrifugation at 35,000 r.p.m. for 90 min. The virion pellet was resuspended in TS buffer and the virions were banded in a CsCl gradient at 1.36 g ml⁻¹. The band containing the virus was collected and, after dilution with TS buffer, the virions were pelleted at 35,000 r.p.m. for 2.5 h. The final pellet was resuspended in TS buffer and the virions were disrupted by adding SDS to 0.5%. The viral DNA was extracted with phenol, precipitated with alcohol and resuspended in 10 mM Tris, pH 7.8, and 1 mM EDTA. Unrestricted DNA was electrophoresed in a 1% agarose gel and restricted DNA in a 0.7% agarose gel in E buffer¹⁶, at 5 V cm⁻¹. The DNA was visualized by staining the gel with ethidium bromide and by photography under UV light. Conditions of restriction are as in Fig. 2 legend.

(Fig. 1*a, b*). This suggested that BAPV has a genome of lower molecular weight (MW) than BCPV. BAPV is cut at one site by *Bam*HI restriction endonuclease and this linear molecule shows a higher electrophoretic mobility than BCPV DNA linearized by *Hind*III (Fig. 1*c, d*). The MW of DNA of BAPV in the above experiments was calculated as approximately 4.5×10^6 , or 10% smaller than the normally quoted MW for papillomavirus DNA^{6,7}.

We have analysed BAPV DNA with six restriction enzymes and compared it with physical maps generated in a similar fashion which we have made of BCPV DNA, and also with DNA from virus isolated from a group of tumours commonly found on the teats of cows and the penis of bulls that we have designated bovine paragenital papillomavirus (BPPV)⁵.

Because of the limited amount of viral DNA obtainable from single alimentary tract papillomas, we found it inconvenient and wasteful to analyse restriction patterns by ethidium bromide fluorescence. We circumvented this problem by labelling some of the virus DNA by nick-translation⁸ and by hybridizing the radioactive DNA to Southern blots⁹ containing nanogramme amounts of restricted DNA. The results are presented in Fig. 2. *Bam*HI and *Hpa*I produce unit length linear DNA (Figs 1*d, 2a, h*); therefore, there is only one cleavage site for these enzymes in the DNA. *Eco*RI produces two fragments (Fig. 2*f*), *Hind*III three fragments (Fig. 2*d*) and *Hind*II four fragments (Fig. 2*b*). The viral DNA is not restricted by *Bgl*II (not shown) and therefore has no cleavage site for this enzyme.

The MWs of the restriction fragments are listed in Table 1. The total MW of the viral genome obtained by adding the MWs of individual restriction fragments generated by any one enzyme ranges from 4.43×10^6 to 4.5×10^6 , thus confirming our previous estimate.

When the DNA is digested with *EcoRI* + *Bam*HI, the *EcoRI* fragment B disappears and two smaller fragments are produced (Fig. 2g). The *Bam*HI site therefore maps within *EcoRI* fragment B. Restriction with *Hind*III + *Bam*HI produces three detectable fragments (Fig. 2e). The first two correspond to *Hind*III fragment A and fragment B, respectively, and the third fragment corresponds to *Hind*III fragment C minus a low MW fragment that we could not detect in this particular gel. The *Bam*HI site is thus located within *Hind*III fragment C. When the DNA is restricted with *Hind*II + *Bam*HI, five fragments are produced (Fig. 2c). Three fragments correspond, respectively, to *Hind*II fragments, B, C and D. *Hind*II fragment A is split into a major fragment and a very low MW fragment. This places the *Bam*HI site within *Hind*II fragment A. Restriction with *Hpa*I + *Bam*HI produces two fragments (Fig. 2i). As the recognition sites of *Hpa*I are a subset of those of *Hind*II¹⁰, the double restriction shows that the single *Hpa*I site coincides with the *Hind*II site at 0.278 map units (see below and Fig. 3). Restriction with *EcoRI* + *Hind*III, and *EcoRI* + *Hind*II places the two *EcoRI* sites within fragments A and C of both *Hind* enzymes (Table 1). Of the three *Hind*III sites, two map within *Hind*II fragment A and one within *Hind*II fragment B (Table 1).

The MWs of the fragments obtained in all double digest experiments are listed in Table 1. Arbitrarily assigning the zero map unit to the single *Bam*HI site, and taking into account the fractional length of all the restriction fragments, these can be ordered in a circular map (Fig. 3).

This map differs considerably from the restriction maps of papillomavirus DNA from both the typical cutaneous warts and the paragenital (teat and penis) lesions⁵ (M.S.C. *et al.*, in preparation). The differences between the viruses are not confined to the position of the restriction sites along their genomes: in liquid hybridization experiments, carried out in 2×SSC at 70 °C, BAPV DNA failed to cross-hybridize to the DNA of the other two viruses, whereas the latter cross-reacted with each other to at least 55% as estimated from the S₁ nuclease resistance of the hybrids. Moreover, BAPV DNA did not hybridize to Southern blots of large amounts (up to 0.5 µg) of restricted BCPV DNA or restricted BPPV DNA, and conversely, neither of the latter hybridized to blots of *Hind*II- and *Hind*III-restricted BAPV DNA. These results show that the alimentary tract papillomavirus is not a deletion mutant of the other two viruses, but belongs to a type of its own.

In contrast, the extensive cross-hybridization between the typical BCPV DNA and BPPV DNA and the respective restriction fragments immobilized on nitrocellulose showed that the sequence homology between these two types of virus is not confined to a particular region of the genome, but is spread throughout the whole of it⁵. Similar results have recently been obtained by Law and colleagues¹¹.

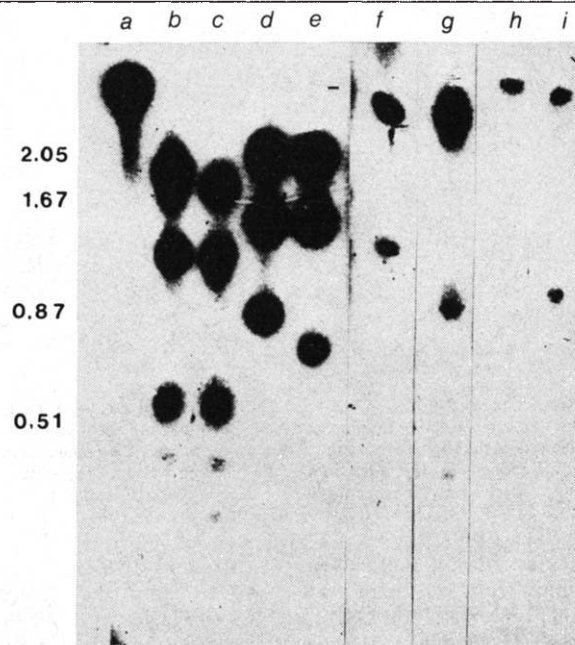


Fig. 2 Restriction fragments of BAPV DNA. a, *Bam*HI; b, *Hind*II; c, *Hind*II + *Bam*HI; d, *Hind*III; e, *Hind*III + *Bam*HI; f, *EcoRI*; g, *EcoRI* + *Bam*HI; h, *Hpa*I; i, *Hpa*I + *Bam*HI (all Boehringer). The buffers used were as recommended by Boehringer laboratories. Nanogramme amounts of viral DNA were digested and the resulting fragments were electrophoresed in a 1.5% agarose gel as described in Fig. 1 legend. The DNA fragments were transferred to nitrocellulose⁹ and hybridized overnight to nick-translated⁸ ³²P-labelled viral DNA, in Denhart-2×SSC at 70 °C (ref. 17). The nitrocellulose sheet was extensively washed in 1×SSC at 70 °C, dried and exposed to a flash-activated X-ray film (Kodak X-Omat H) at -70 °C (ref. 18) for 3 weeks. The *Hind*II fragments of BCPV DNA, visualized both by ethidium bromide fluorescence and by hybridization to ³²P-BCPV DNA, were used as MW markers. The sharpness of the stronger high MW bands has been lost so that the presence of the much fainter low MW bands can be seen.

Marked differences between BAPV on the one hand and BCPV and BPPV on the other were also found at the level of antigenic determinants. Whereas BCPV and BPPV have a major determinant in common, no serological cross-reaction was observed with BAPV⁵ (W.F.H.J. *et al.*, in preparation). Thus, BCPV and BPPV seem to belong to the same group and, although distinguishable, are much more closely related to each other than they are to BAPV.

Two types of bovine papillomavirus, BPV 1 and BPV 2, have recently been described¹². Although the precise body localization of the papillomas from which these viruses were purified is not specified in that work, BPV 1 corresponds to our BPPV and BPV 2 to our BCPV. Also, bovine papillomavirus with a genome of MW 4.5 × 10⁶ has recently been found by Pfister *et*

Table 1 Molecular weights and fractional lengths of the restriction fragments of BAPV DNA

MW (×10 ⁻⁶)	Fractional length	MW (×10 ⁻⁶)	Fractional length	MW (×10 ⁻⁶)	Fractional length	MW (×10 ⁻⁶)	Fractional length	MW (×10 ⁻⁶)	Fractional length
<i>EcoRI</i>		<i>Hind</i> III		<i>Hind</i> II		<i>EcoRI</i> + <i>Bam</i> HI		<i>Hind</i> III + <i>Bam</i> HI	
A 3.15	70.0	A 2.13	47.4	A 2.10	47.9	3.14	70.0	2.13	47.4
B 1.35	30.0	B 1.48	32.9	B 1.30	29.6	1.00	22.1	1.48	32.9
4.50		C 0.88	19.6	C 0.56	12.8	0.36	8.0	0.70	15.6
		4.49		D 0.42	9.5	4.50		4.31	
				4.38					
<i>Hind</i> II + <i>Bam</i> HI		<i>Hind</i> III + <i>EcoRI</i>		<i>Hind</i> II + <i>EcoRI</i>		<i>Hind</i> II + <i>Hind</i> III		<i>Hpa</i> I + <i>Bam</i> HI	
1.82	41.5	1.48	33.2	1.52	34.7	1.32	29.8	3.3	73.3
1.30	29.6	1.30	29.2	1.30	29.6	1.22	27.3	1.2	26.6
0.56	12.8	0.79	17.7	0.60	13.7	0.88	19.9	4.5	
0.42	9.6	2 × 0.44	9.9	0.42	9.5	0.56	12.7		
0.26	5.9	4.45				0.43	9.7		
4.36									

The MWs were calculated as in Fig. 2 legend. In two different experiments, the measurement error ranged from 0.01 × 10⁶ to 0.15 × 10⁶ for individual fragments. The fractional lengths were calculated from the percentage of the MW of a single fragment over the total MW. In the case of *Hind*II + *EcoRI* double digest and of *Hind*II + *Hind*III double digest, where small fragments were missing, presumably because they failed to bind to the nitrocellulose, the fractional length was calculated on the total MW of the single digest.

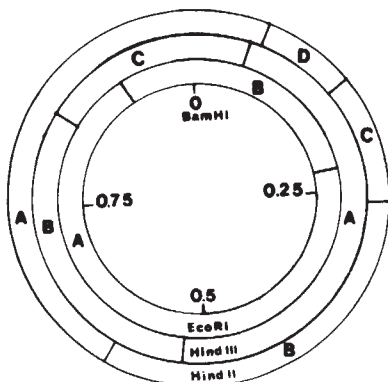


Fig. 3 Restriction map of BAPV DNA. The circular genome has been divided into tenths, and the restriction enzyme sites have been mapped according to the fractional length of the fragments produced by a given enzyme. Zero map unit has been arbitrarily assigned to the single *Bam*HI site. The approximate map positions of the other restriction sites are: *Hpa*I = 0.278; *Eco*RI = 0.220, 0.920; *Hind*III = 0.040, 0.514, 0.844; *Hind*II = 0.057, 0.152, 0.278, 0.587

*al.*¹³ in cutaneous papillomas from Australian cattle, but not in papillomas from German cattle where only the typical cutaneous type was found. Despite the similarity in MW, the DNA restriction map of the Australian virus found by those authors differs from the DNA restriction map of the small virus we have found in alimentary tract papillomas. Experiments in progress suggest that 'rice grain'-type lesions of the teat papillomatosis are caused by yet another type of papillomavirus. This would bring the number of different bovine papillomaviruses to five. We are now investigating the relationship between the several viruses at the molecular, immunological and pathological levels. We injected BAPV into the soft palate of 18 calves and into the skin of the neck of 6. After 2 months, all 18 calves injected in the palate showed marked papillomatosis, whereas no cutaneous papillomas have so far (8 months) been detected in the calves injected subcutaneously. Studies on the infectivity of BAPV are in progress. The viral DNA purified from the experimentally induced alimentary tract papillomas is indistinguishable in MW and restriction pattern from the one used to induce the tumours. In addition, the sera from the tumorous calves failed to cross-react with antisera raised against the other viruses, confirming the uniqueness of BAPV. The finding that BAPV does not induce cutaneous warts complements the finding of Olson and his colleagues¹⁴, who, although able to induce cutaneous warts experimentally in cattle with BCPV, failed to infect the alimentary tract.

Thus, although our analysis of these viruses is incomplete, our initial studies of their epidemiological, pathological, immunological and molecular heterogeneity provide increasing evidence of a close relationship between the type of papillomavirus and the type and site of lesion caused by them. This is particularly interesting in view of the fact that, as far as we know, only BAPV is associated with the only benign papillomas that commonly undergo malignant transformation in cattle. A similar situation is found in humans: epidermodysplasia verruciformis, a papilloma with marked tendency to malignant transformation, is caused by HPV-5 (previously classified as HPV-4), a human papillomavirus differing from the other, more common, viruses associated with ordinary skin warts¹⁵. In addition, preliminary evidence indicates that bovine alimentary carcinoma cells contain BAPV DNA sequences, but not BCPV or BPPV DNA sequences, thus further suggesting that only the alimentary virus is involved in carcinogenesis.

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A pyrimidine dimer-DNA glycosylase activity associated with the *v* gene product of bacteriophage T4

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Mutations in the *v* gene of bacteriophage T4 are associated with a marked increase in sensitivity to killing by UV radiation at 254 nm, but not to a variety of other forms of base damage to DNA¹⁻⁴. Early studies from this laboratory provided evidence for a role of the *v* gene in the excision of pyrimidine dimers (PD) from DNA. Specifically, it was shown that extracts of T4_v⁺-infected *Escherichia coli* catalyse the formation of single-strand breaks (nicks) and/or alkali-labile sites in UV-irradiated duplex DNA⁵. Comparable hydrolysis of phosphodiester bonds is not observed with extracts of *E. coli* infected with the mutant T4_v₁ (ref. 5). The product of the *v* gene has been extensively purified in a number of laboratories⁶⁻⁹; however, convincing evidence of purification to physical homogeneity has not yet been presented.

Characterization of the partially purified *v*-gene product yielded results that were consistent with the interpretation that this gene codes for an endonuclease activity (endonuclease V of T4 or T4 UV endonuclease) that directly catalyses the hydrolysis of phosphodiester bonds 5' to PD in duplex DNA, creating nicks with 3'OH and 5'P termini^{6,8,10,11}. The additional observations that the enzyme attacks single-stranded DNA containing PD^{6,11} and that no other form of base damage in DNA thus far examined is recognized as substrate^{1-4,12}, support the notion that the activity coded by the *v* gene recognizes PD in DNA specifically.

Grossman *et al.*¹³ and Haseltine *et al.*¹⁴ have recently suggested an alternative mechanism for the incision of DNA containing PD. Their suggestion stems from the analysis by gel electrophoresis, of the length of DNA fragments generated by incubation of a sequenced region of the *E. coli lac* operon containing PD, with UV endonuclease activities from *Micrococcus luteus*. A knowledge of the exact nucleotide sequence of the substrate used allowed for a very precise prediction of the length of the oligonucleotide products generated following denaturation of the DNA, if the enzyme activity catalysed cleavage of phosphodiester bonds immediately 5' to PD sites. Their experiments yielded unexpected results which have been interpreted in terms of an alternative molecular mechanism for the enzymatic incision of UV-irradiated DNA at PD sites, that is, a PD-specific DNA glycosylase catalyses cleavage of the 5' N-glycosylic bonds of the dimerized pyrimidines and the resulting apyrimidinic sites are attacked by apurinic/apyrimidinic (AP) endonuclease, resulting in cleavage of phosphodiester bonds. This two-step mechanism is entirely consistent with the observation of nicks bearing 3'OH and 5'P termini located 5'

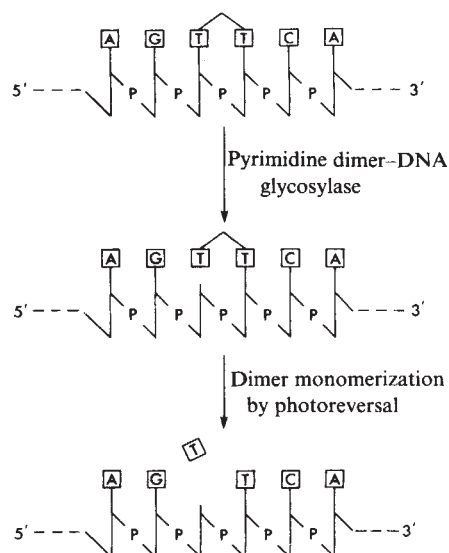


Fig. 1 Diagrammatic representation of experimental protocol. Only one strand of a duplex DNA molecule is shown. The figure indicates postulated hydrolysis of the 5' glycosidic bond of a thymine-thymine dimer, yielding an apyrimidinic site. Subsequent monomerization of the dimer by direct photoreversal results in the release of the 5' thymine of the dimer as free thymine.

with respect to PD in duplex UV-irradiated DNA incubated with *M. luteus* correndonucleases I and II (ref. 15).

In view of the remarkable similarity in the properties of so-called T4 endonuclease V and *M. luteus* correndonucleases I and II, we have sought evidence for a PD-DNA glycosylase activity in preparations of T4 enzyme known to catalyse the nicking of UV-irradiated DNA. Enzyme-catalysed hydrolysis of the *N*-glycosidic bond linking either one of the dimerized pyrimidines to deoxyribose in DNA would leave the corresponding base in covalent linkage only with its partner in the dimerized structure (Fig. 1). Subsequent monomerization of the PD should result in the liberation of free thymine (Fig. 1) which can be readily detected by established chromatographic techniques. Our experimental approach has been to demonstrate that the formation of free thymine from UV-irradiated DNA is dependent both on incubation with T4 enzyme activity, and on the monomerization of PD by the technique of direct photoreversal.

Regarding the latter procedure, it has been clearly demonstrated by others that the formation of pyrimidine dimers in DNA by exposure to UV radiation at about 254 nm is a reversible process^{16,17}. When *E. coli* DNA is irradiated at 254 nm under standard conditions, the rates of PD formation and reversal ultimately become equal and a state of dynamic equilibrium is attained in which ~7% of radioactively labelled thymine is in PD. Thus, further irradiation at 254 nm under these conditions does not result in measurable net dimer formation or dimer monomerization. However, when *E. coli* DNA is initially irradiated at 254 nm in the presence of AgNO₃, photosensitized dimer formation results in the conversion of as much as 20% of the thymine to thymine-containing PD¹⁸. Further irradiation of this DNA at 254 nm following removal of the photosensitizer results in measurable reversal of PD, until once again a steady state is reached at a level of ~7% thymine-containing PD. In the present experiments we have employed the technique of direct photoreversal to investigate the release of free thymine from *E. coli* DNA (containing about 17% of its thymine in PD) previously incubated with or without a T4 enzyme preparation.

Figure 2 shows the sedimentation profiles in alkaline sucrose gradients of UV-irradiated DNA incubated with the T4 enzyme. It is clear that the smallest amount of enzyme used in these experiments is saturating with respect to the number of DNA strand breaks and/or alkaline labile sites generated in the DNA. Unirradiated DNA incubated and sedimented under identical

conditions showed no reduction in sedimentation velocity in alkaline sucrose gradients (data not shown). The incubation conditions described in Fig 2 also result in the release of free thymine. (Table 1 and Fig. 3). However this product is only observed when, following incubation of UV-irradiated DNA with the T4 enzyme preparation, the reaction mixture is subjected to direct photoreversal. Free thymine is not detected when unirradiated DNA incubated with enzyme is subjected to photoreversing light (Table 1), nor is this result obtained when UV-irradiated DNA not previously incubated with the enzyme is exposed to photoreversing light (Table 1).

These results strongly suggest that the T4 enzyme preparation contains a DNA glycosylase activity that catalyses the hydrolysis of one of the *N*-glycosidic bonds linking the dimerized pyrimidines to deoxyribose in DNA (Fig. 1). This suggestion is consistent with the fact that PD are quantitatively the major photoproduct in DNA irradiated at 254 nm, and to our knowledge are the only known photoproducts in DNA that can generate thymine as a product of their photolabilization. Our conclusions are also supported by the results of extensive previous studies with the T4 enzyme which have demonstrated that this activity recognizes pyrimidine dimers exclusively in UV-

Table 1 Release of free thymine from DNA containing thymine dimers

³ H-labelled thymine-labelled substrate	T4 enzyme	Incident photo-reversal fluence (kJ m ⁻²)	Total free [³ H] thymine detected (c.p.m. per ml of reaction mixture × 10 ⁻³)	(% of [³ H] radioactivity as thymine-containing PD)
Unirradiated <i>E. coli</i> DNA	Absent	0	<0.04	<0.001
	Absent	11.5	<0.04	<0.001
	Present	0	<0.04	<0.001
	Present	11.5	<0.04	<0.001
UV-irradiated <i>E. coli</i> DNA (17.5% thymine as thymine-containing PD)	Absent	0	<0.04	<0.001
	Absent	4.3	ND	—
	Absent	8.2	ND	—
	Absent	12.3	<0.04	<0.001
	Present	0	<0.04	<0.001
	Present	4.3	14.4	16.5
	Present	8.2	21.3	24.3
	Present	12.3	26.6	30.4

[³H]thymine-labelled *E. coli* DNA (1.5 × 10⁴ c.p.m. μg⁻¹) was prepared by standard methods. DNA containing 17.5% of ³H-thymine in PD was produced by germicidal irradiation at 254 nm with an incident fluence of 2 kJ m⁻² in the presence of Ag⁺ photosensitizer according to the procedure of Rahn and Landry¹⁸. Tritiated UV-irradiated or unirradiated DNA (100 μg ml⁻¹) was incubated at 37 °C with or without 9.4 × 10⁴ units ml⁻¹ of the phosphocellulose fraction of T4 endonuclease⁹ in 10 mM Tris-HCl pH 7.8, 10 mM Na₂EDTA, 100 mM NaCl for 45 min in a total volume of 3.0 ml. Following incubation, each was deproteinized by incubation at 60 °C with 50 μg per ml Proteinase K for 1 h followed by extraction with an equal volume of CHCl₃. Irradiated or unirradiated ³H-DNA (131 μg total) that had been incubated in the presence or absence of enzyme was diluted with 10 mM Tris-HCl pH 7.5, 1 mM Na₂EDTA to give a final activity of 5.0 × 10⁵ ³H c.p.m. ml⁻¹. Unlabelled DNA was added to these mixtures to bring the final A₂₅₄ to 2.0. The mixtures were then subject to photoreversing irradiation and duplicate aliquots (0.2 ml) were withdrawn at the incident fluences stated. [¹⁴C]-thymine (9,530 c.p.m. NEN) was added to each aliquot as an internal marker for the chromatographic identification of free ³H-thymine by TLC. The aliquots were evaporated to dryness and the residue extracted three times with 15 μl absolute ethanol. The extracts were spotted directly on thin layer plates and developed as described previously²². [³H] and [¹⁴C] radioactivity were measured in individual chromatogram fractions; total [³H] free thymine was calculated by normalization to 100% recovery of the [¹⁴C] standard. Separate [¹⁴C]-thymine samples were run with each chromatogram to allow for correction of channel spillover during radioactive counting of the two isotopes in a Beckman LS 250 liquid scintillation spectrometer. Replicate measurements agreed to within 5%. (ND = none detected.)

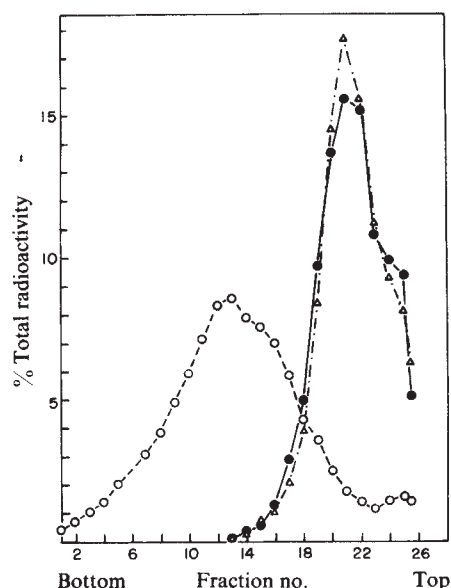


Fig. 2 ^3H -thymine DNA ($0.5\ \mu\text{g}$, 21% thymine-containing PD) was prepared as described in the legend to Table 1 and incubated at 37°C for 30 min with (●) or without (○) 9.4×10^4 units per ml of the phosphocellulose fraction of T4 endonuclease⁹ in $50\ \mu\text{l}$ reactions containing 10 mM Tris-HCl pH 7.8, 10 mM Na_2EDTA , 100 mM NaCl. The reactions were stopped by the addition of $50\ \mu\text{l}$ 5% alkaline sucrose, and layered onto 4 ml 5–20% alkaline sucrose gradients. The DNA was sedimented at 30,000 r.p.m. in an SW56 rotor for 11 h at 20°C . The gradients were fractionated and processed for measurement of radioactivity as described by Reynolds²³. To demonstrate apparent completion of incision, a reaction containing enzyme was prepared and incubated as above, and then incubated with an additional aliquot (4.7×10^3 units) of enzyme for a further 30 min (Δ). Incubations with even higher amounts of enzyme yielded identical sedimentation profiles (data not shown).

irradiated DNA^{2,7,11,19–21}. Thus, for example, removal of PD by enzymatic photoreactivation of UV-irradiated DNA in permeabilized *E. coli* results in the loss of all sites sensitive to the T4 enzyme²⁰. In addition, in both duplex and single stranded UV-irradiated DNA incubated with the enzyme there is a stoichiometric relationship between the calculated number of PD in the DNA and the measured number of strand breaks^{7,11,19,21}. Further evidence in support of PD-DNA glycosylase activity associated with the T4 enzyme preparation is provided by independent studies by Seawell *et al.* (manuscript submitted). Using the same preparation of T4 enzyme, these investigators have shown that under incubation conditions that are strictly limiting with respect to enzyme concentration, UV-

Table 2 Photoreversal-dependent release of free thymine from UV-irradiated DNA incubated with extracts of *E. coli* infected with phage T4v⁺ or T4v₁

Extract of <i>E. coli</i> infected with	Free thymine (c.p.m./20 μl of reaction mixture)	
	Photoreversal	No photoreversal
Phage T4v ⁺	380	<20
Phage T4v ₁	<20	<20

Extracts of *E. coli* cells infected by wild type (v^+) and mutant (v_1) phage T4 were prepared by sonication in 50 mM Tris-HCl, pH 8.0 as described previously⁷. Extracts (0.87 mg total protein in each) were incubated at 37°C for 30 min with $150\ \mu\text{g}$ ^3H -thymine labelled *E. coli* DNA (3.0×10^4 c.p.m. per μg , 10% of [^3H] in thymine-containing PD) plus 35 mM Tris-HCl pH 8.0, 10 mM Na_2EDTA , 100 mM NaCl. The final volume of each of the two incubation reactions was 1.6 ml. Following reaction, the mixtures were extensively deproteinized by incubation with 100 μg per ml Proteinase K at 60°C for 2 h, followed by extraction with phenol and CHCl_3 . The mixtures were made 0.5 M in NaCl and the DNA was precipitated overnight at -20°C with two volumes of absolute ethanol. The DNA was collected by centrifugation and resuspended at a concentration of $150\ \mu\text{g}\ \text{ml}^{-1}$ in 10 mM Tris-HCl pH 7.5, 1 mM EDTA. Half of each reaction mixture was subjected to $10\ \text{kJ}\ \text{m}^{-2}$ photoreversal irradiation fluence; the other half was kept as a control. Aliquots (20 μl) of each reaction were spotted on thin layer chromatography plates. Free thymine was determined as described in the legend to Table 1. [^{14}C] thymine standard samples were run on adjacent lanes of the same thin-layer plate.

irradiated DNA is subject to hydrolysis of phosphodiester bonds in strong alkali or following incubation with AP endonuclease at near-neutral pH. These are expected properties of DNA containing apyrimidinic sites generated by the action of a DNA glycosylase activity. Alkaline lability of T4 enzyme-treated UV-irradiated DNA and/or the presence of AP endonuclease activity associated with the T4 DNA glycosylase is also indicated in the present studies by the reduced sedimentation velocity of the DNA in alkaline sucrose gradients shown in Fig. 2.

Note from Table 1 that the highest photoreversing fluence used in these experiments ($12\ \text{kJ}\ \text{m}^{-2}$) is not yet saturating with respect to free thymine formation. Yet even at this fluence, the amount of radioactivity present in the free base is equivalent to that present in $\sim 30\%$ of the thymine-containing PD measured in the DNA. Since the DNA is uniformly labelled with ^3H -labelled thymine, each thymine-containing dimer attacked by the enzyme and subsequently reversed by irradiation, should release on the average one half of its radioactivity as free thymine (Fig. 1). Thus the radioactivity in free thymine equivalent to 30% of the thymine-containing PD in DNA represents the product of the reversal of 60% of such dimers. Clearly the PD-DNA glycosylase demonstrated in these experiments is not a minor contaminant of the T4 enzyme preparation used.

Finally, our studies indicate that the T4 PD-DNA glycosylase is a product of the *v* gene of phage T4, since extracts of T4-infected *E. coli* failed to catalyse the production of free thymine under the experimental conditions described (Table 2). A question of obvious interest is whether or not the *v* gene also codes for an AP endonuclease activity that is physically associated with the PD-DNA glycosylase activity. The release of free thymine dependent on the photoreversal of PD in DNA provides the basis for a specific and convenient assay of T4-induced PD-DNA glycosylase activity and we are currently purifying this enzyme to physical homogeneity from T4-infected

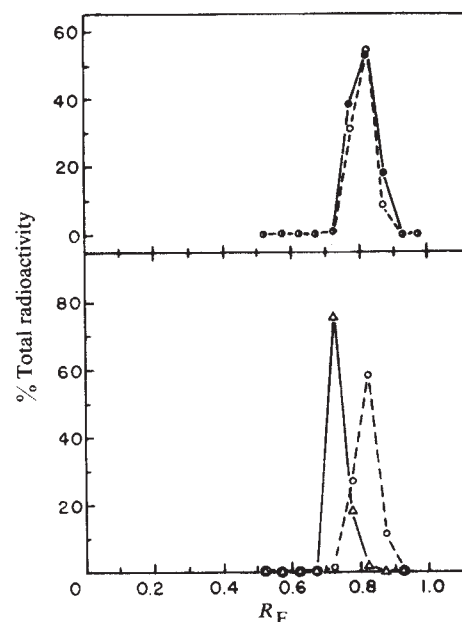


Fig. 3 Chromatographic identification of free ^3H -thymine in the ethanol-soluble phase generated by photoreversal irradiation of T4-enzyme treated irradiated DNA. Upper panel: portions of the ethanol-soluble phase of the photoreversal reaction containing added ^{14}C -thymine were applied to silica gel thin-layer chromatography plates and developed with water-saturated ethyl acetate/*n*-propanol (4:1)¹⁷. The plates were then fractionated at 0.05 R_f increments and the radioactivity in each fraction was determined after elution into water by liquid scintillation spectrometry²². Phosphorylated species migrate in this system with an $R_f = 0.1$ (ref. 17). Lower panel: ^3H -thymidine and ^{14}C -thymine were mixed and applied to an adjacent lane of the same plate depicted in the upper panel and analysed identically following development. No radioactivity migrated with an $R_f < 0.5$ in either chromatogram (data not shown). ●, [^3H] reaction product; Δ, [^3H]TdR standard; ○, [^{14}C]T standard.

E. coli. The availability of purified enzyme will hopefully facilitate a more detailed examination of the relationship of PD-DNA glycosylase to AP endonuclease activity, and of other questions concerning the detailed molecular mechanism of the incision of UV-irradiated DNA by this enzyme.

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Calcium-induced decrease in membrane fluidity of sea urchin egg cortex after fertilization

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Fertilization of the sea urchin egg is a dramatic example of cell activation resulting from the interaction of an external stimulus, the spermatozoon, with the cell surface¹. Growing and quiescent cells may have different membrane states². Here we report membrane fluidity measurements on a surface membrane fraction, the cortex, isolated from unfertilized and fertilized eggs. The fluidity of the fertilized egg cortex, measured by electron spin resonance (ESR) spectroscopy using 5-doxylstearate as a probe, is less than that of the unfertilized cortex. In the intact egg the intracellular Ca^{2+} concentration increases after fertilization and this transient rise seems to be localized in the cortex³. The addition of Ca^{2+} to the cortex fraction isolated from unfertilized eggs triggers a fluidity decrease *in vitro*. The fluidity decrease seems to represent a Ca^{2+} -induced change in membrane structure rather than a direct interaction of Ca^{2+} with phospholipid headgroups.

Bulk membrane fluidity of sea urchin eggs increases after fertilization or partial activation by ammonia^{4,5}. We could not determine the effect of fertilization on plasma membranes of intact eggs because 5-doxylstearate probably partitions throughout egg membranes⁶ and plasma membrane accounts for only a small percentage of the membrane present. The egg cortex can be isolated in a functional form^{6–9} (see Table 1 legend for details). The cortex fraction contains several components in addition to plasma membrane. The extracellular vitelline layer of the unfertilized egg becomes the fertilization envelope after fertilization¹⁰. The hyaline layer surrounding the fertilized egg cortex holds the blastomeres together¹¹. Cortical granules, membrane-delimited vesicles ~1 μm in diameter, line the inner surface of the plasma membrane of unfertilized eggs and fuse with the plasma membrane within seconds of fertilization¹⁰. The addition of Ca^{2+} to the unfertilized egg cortex triggers the discharge of cortical granules *in vitro*^{6,10}, a process analogous to the cortical granule exocytosis which occurs as the intracellular Ca^{2+} level rises after fertilization³. When viewed by scanning electron microscopy or phase contrast microscopy, our cortex fraction consisted of nearly intact cortical hulls and smaller, morphologically unidentifiable membrane fragments. On addition of Ca^{2+} to the unfertilized egg cortex fraction the cortical granules disappeared and each cortical fragment twitched and became less opaque by phase contrast microscopy. By this visual assay our cortex preparation was apparently more than 90% pure. The purification of plasma membrane in this fraction was at least 10-fold, as judged by the specific activities of mitochondrial and endoplasmic reticulum enzyme markers, and nucleic

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Table 1 Enzyme and nucleic acid content of cortex fractions

Sample	Cytochrome c oxidase specific activity		NADH oxidase specific activity		Nucleic acid protein (w/w)
	U per mg protein per min	Relative	U per mg protein per min	Relative	
Unfertilized egg homogenate	1.38	(100%)	0.48	(100%)	0.135
Unfertilized egg cortex	0.092	6.5	0.056	11.6	0.012
Fertilized egg homogenate	1.33	(100%)	0.65	(100%)	0.128
Fertilized egg cortex	0.11	8.3	0.083	12.7	0.013

Enzyme activities, protein and DNA content were measured for cortex fractions isolated from unfertilized and fertilized *Strongylocentrotus purpuratus* eggs. The procedures used were modifications of the methods described by Schatten and Mazia⁷ and Grainger⁹. The samples were maintained at 15 °C throughout the isolation. The jelly coat was removed by incubating eggs for 2 min in seawater (Instant Ocean, Aquarium Systems) titrated to pH 5 with HCl. Dejellied, unfertilized eggs were washed in isolation medium containing (mM): glycine, 350; NaCl, 300; MgCl_2 , 4; EGTA, 10; Tris base added to a final pH of 7.6. The eggs were hand-homogenized (8–20 strokes) in a Teflon-glass homogenizer in isolation medium containing 0.2 mg ml^{-1} soybean trypsin inhibitor. Cortices were collected by centrifugation at 700g for 10 s, washed once with isolation medium–soybean trypsin inhibitor, then washed twice with isolation medium. For cortex isolation from fertilized eggs, 3 volumes of 10 mM dithiothreitol in artificial seawater titrated to pH 9.1 with Tris base were added to dejellied, fertilized eggs 20 s after addition of sperm. This treatment dissolved the fertilization envelope²⁰. After 4 min, 9 volumes of Ca^{2+} -free seawater were added with stirring. Ca^{2+} -free seawater contains (mM): NaCl, 460; MgCl_2 , 55; KCl, 10; NaHCO_3 , 2.5; EGTA, 10; titrated to pH 8.0 with Tris base. After 10 min stirring, the eggs were allowed to settle, then washed with isolation medium and homogenized as described above. Protein and nucleic acid were assayed spectrophotometrically²¹. Cytochrome c oxidase and NADH oxidase were assayed as described elsewhere^{22,23}.

acid content (Table 1). From the data of Schroeder¹² we estimate that 50% of the membrane surface in the cortex fraction is plasma membrane and 50% cortical granule membrane. The purity of plasma membrane in the cortex fraction compares favourably with that of other purified plasma membrane fractions from animal cells despite the presence of cortical granules.

We measured membrane fluidity in the cortex fraction isolated from fertilized and unfertilized eggs. The order parameter, *S*, derived graphically from the ESR spectrum of 5-doxylstearate, decreases from 1 to 0 as the system becomes less ordered¹³. 5-Doxylstearate does not reside in neutral lipid droplets in these conditions⁵, and the rapid, anisotropic motion revealed in the ESR spectrum means that 5-doxylstearate is probably not bound to protein⁵. Thus, an increase in order parameter may be interpreted as a decrease in membrane fluidity⁵. *S* measured for fertilized egg cortices isolated 30 min after fertilization is $3.2 \pm 1.3\%$ greater than when measured for unfertilized cortices from the same batch of eggs (Table 2). This change is about 50% greater than the change in *S* of bulk membranes^{4,5}, and is in the opposite direction. The response of this surface membrane fraction to fertilization seems to be distinct from the response of intracellular membranes.

An increase in *S* also accompanied the *in vitro* discharge of cortical granules of the unfertilized egg cortex (Table 3). In expts 1–5, cortical granules were discharged by adding a sufficient quantity of 0.1 M CaCl₂ just to titrate the 10 mM EGTA present in the isolation medium. The average ΔS observed for calcium addition *in vitro* was about the same as the average ΔS observed in the cortex fraction after fertilization. For example, the average % ΔS measured at ambient temperature for unfertilized relative to fertilized egg cortices was 3.4 ± 1.8 compared with an average % ΔS of 3.3 ± 0.6 for the change induced by addition of Ca²⁺, measured at ambient temperature. The cortical granules of unfertilized egg cortices spontaneously discharge in isolation medium lacking EGTA. Spin labelled cortices allowed to undergo spontaneous discharge after removal of EGTA also exhibited an increase in *S* (expt 6). Mild heat treatment in the presence of EGTA discharges cortical granules. Cortices treated for 2 min at 40 °C in isolation medium containing 10 mM EGTA exhibited a similar increase in *S* (expt 7).

Table 2 Order parameter of cortices isolated from unfertilized and fertilized eggs

Expt	Temperature	<i>S</i> unfertilized cortex	<i>S</i> fertilized cortex	ΔS (%)
1	Ambient	0.669	0.683	2.1
2	Ambient	0.657	0.687	4.6
3	12 °C	0.756	0.777	2.8

Each experiment represents cortices isolated from the eggs of a different animal. 5-Doxylstearate in methanol was dried onto the wall of a test tube under a stream of nitrogen. A cortex suspension (1 ml) (Table 1) was added, the mixture was incubated for 10 min at 15 °C, then a cortex pellet was collected by centrifugation for 10 s at 700 g. The wet cortex pellet was washed with 0.5 ml isolation medium, then transferred to a Pasteur pipette sealed at one end. The molar ratio of spin label added to cortex phospholipid was approximately 1:30. ESR spectra were recorded from samples in sealed Pasteur pipettes on a Varian E-104A or E-4 ESR spectrometer equipped with a Varian temperature control accessory. Microwave power was 10 mW and modulation amplitude was 1 or 2 G. *S* was measured graphically from the spectra¹³. Multiple measurements of these samples indicated that the accuracy of the % ΔS measurement is $\pm 0.5\%$.

The exocytosis of cortical granules seems to be self-propagating *in vivo*¹⁰ and *in vitro*⁶ and a release of Ca²⁺ from the cortex may be involved³. Ca²⁺ interacts with the polar head group of phospholipids, rendering them less fluid¹⁴, but that is an unlikely mechanism for the effects described here. The intrinsic association constant of Ca²⁺ for negatively charged phospholipids is

Table 3 Effect of *in vitro* cortical granule discharge on order parameter of unfertilized egg cortex

Expt	Method of discharge	Temperature (°C)	<i>S</i> before discharge	<i>S</i> after discharge	ΔS (%)
1	Add CaCl ₂	Ambient	0.595	0.617	3.7
2	Add CaCl ₂	Ambient	0.591	0.608	2.9
3	Add CaCl ₂	12	0.727	0.748	2.9
4	Add CaCl ₂	12	0.771	0.818	6.1
5	Add CaCl ₂	12	0.753	0.776	3.0
6	Remove EGTA	12	0.759	0.790	4.1
7	Heat	12	0.747	0.800	7.1

Each experiment represents cortices isolated from a different animal. Measurements were made as described in Table 2 legend. CaCl₂ was added to washed, labelled cortices as 0.5 M CaCl₂–0.5 M Tris base. The pH after Ca²⁺ addition was 7.6 ± 0.1 . EGTA was removed by washing with isolation medium lacking EGTA. Heat discharge was effected by warming the sample to 40 °C for 2 min. Multiple measurements on these samples indicated that the accuracy of the $\Delta S\%$ measurement is $\pm 0.5\%$.

of the order of 10 M^{-1} (S. McLaughlin, unpublished results). We estimate that the Ca²⁺ concentration is less than $1 \mu\text{M}$ for all the samples studied here except those lacking EGTA or containing added Ca²⁺. The intracellular Ca²⁺ concentration was estimated to reach $2.5\text{--}4.5 \mu\text{M}$ after fertilization³. At these concentrations less than 1% of the negatively charged lipids should carry bound Ca²⁺, even considering that the surface concentration of Ca²⁺ may be 100-fold greater than the bulk concentration because of the effect of the membrane surface potential¹⁵. It seems clear that Ca²⁺ triggers the observed decrease in fluidity of the cortex but it apparently acts through membrane proteins rather than directly on the phospholipid bilayer.

Fluorescence photobleaching recovery measurements on mouse eggs revealed 10–100-fold slower rates of lateral diffusion on the cell surface after fertilization¹⁶. Studies on the rheological properties of the sea urchin egg surface showed that the cortex of the cell stiffens in response to fertilization¹⁷. These results seem consistent with the decrease in fluidity reported here.

These changes in fluidity are sufficient to alter the activity of membrane-bound enzymes such as adenylyl cyclase and (Na⁺ + K⁺)ATPase by several fold (refs 18, 19 and M. Sinensky, unpublished results), assuming that the enzymes undergo the same change in fluidity as does the spin probe. The fluidity change could have an important role in the transition of the membrane from its quiescent to its proliferating state².

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BOOK REVIEWS

The essential Lamarck

R.W. Burkhardt

JUST as it was once common in natural history to view a wide array of species as existing primarily for the benefit of man, so too was it once common in the history of science to consider a large number of scientists as being adequately understood once they were identified as the 'precursors' of later thinkers. It is a mark of the growing maturity of the history of science that the 'precursoritis' that plagued the field as recently as a generation ago has now generally disappeared. French historians of science, most notably Alexandre Koyré and Georges Canguilhem, were prominent among those who argued that precursorship is a concept that is antithetical to the appreciation of scientific ideas in the past. Now Madeleine Barthélemy-Madaule, Professor of Philosophy at the Université de Picardie, has taken the insight of Koyré and Canguilhem and applied it to the case of Jean-Baptiste Lamarck (1744–1829), the French biologist who for about a century has been acclaimed, especially in France, as one of the great precursors of modern evolutionary thought.

Lamarck is a good choice for illustrating the problems of the notion of precursorship. His name is well known, but the ideas for which he is remembered fail to illuminate either the nature of his own work or the scientific problems of his day. Madaule's book is not the first study to make this point. The rethinking of Lamarck's place in the history of science began with papers written by Charles Gillispie in the 1950s and has continued up to the present. It is refreshing, nonetheless, to find that a book published in Lamarck's own country on the 150th anniversary of his death is directed more toward the issue of relocating Lamarck in his own historical context than toward the issues of who deserves the title of 'founder of evolution' or what Darwin owed to Lamarck.

Madaule has not attempted to provide an extensive treatment of Lamarck's life and work. She has sought instead to examine some of Lamarck's essential concepts in order to make clearer the interrelations of the ideological and the scientific elements of Lamarck's thought. At the same time she has sought to put to rest the "myth" of the precursor. As she explains, this is to be achieved through

Lamarck ou le Mythe du Précurseur. By M. Barthélemy-Madaule. Pp.185. (Editions du Seuil: Paris, 1979.) Approx. 55F, £7.

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"the exploration of the possibilities of an epoch, the vigilant notation of the concepts that are utilized and of those that are not" (page 74). After an introductory chapter on Lamarck's life, she successively considers his ideas on nature, the relation between the animal "series" and environmental "circumstances", the inheritance of acquired characters, and adaptation. These are concepts in Lamarck's thought which have often been discussed, but Madaule manages to bring good sense and originality to her analysis of each of them. Her final chapter surveys how Lamarck came to be identified as a precursor of various later thinkers.

The author's treatment of the role of "ideology" in Lamarck's thought is intriguing, but it is surprisingly narrow given her references to Canguilhem's and Althusser's thoughts on ideology in

science. The ideological element she identifies in Lamarck's thinking is a theological one, manifesting itself in Lamarck's concepts of nature and the animal series and the relation of these concepts to Lamarck's thoughts on "the Supreme Author of all things". Madaule's observations on the ambiguities and tensions in Lamarck's presentation of these concepts are excellent and constitute an important contribution to Lamarckian scholarship. However, her reference to the ideological element in Lamarck's thinking as a "survival" (page 76), her view of the progressive elimination of this element along the route from Lamarck's evolutionary theory to Darwin's (page 139) and her phrase "ideological contamination" (page 141) suggest that she is operating with a restricted and generally negative view of the role of ideology in science. It is curious that Lamarck's commitment to a naturalistic explanation of mental as well as physical phenomena does not qualify in some measure as "ideological" and is not subjected to closer historical and philosophical scrutiny.

It is also curious that in a book devoted to placing Lamarck's thought in its own historical context considerable space is given to the issue of whether or not recent developments in molecular genetics have brought back the possibility that acquired characters may indeed be inherited. It would have been more appropriate to devote this space to examining how Lamarck's thinking developed over time, how the concepts selected for treatment here fit in with the rest of his scientific and philosophical thought, or how his views compared with those of his contemporaries. Madaule only scratches the surface of this part of the historical task. Generally speaking, her philosophical orientation makes her more sensitive to the apparent relation of elements within Lamarck's thinking at a given time than to the ways in which his thought was a dynamic, evolving entity. Her discussion of Lamarck's thoughts on species, for example, confuses the structural relations within one of Lamarck's general discourses for the historical situation in which the problem of species change came to be especially crucial for him.

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Madaule acknowledges that history is reconstructed according to the concerns of the historian. What she has tried to offer the reader is "a Lamarck seen in his own epoch through the dimensions of a perspective produced by the contemporary world" (page 175), the perspective in question being that which concerns itself with the interrelations of science and

ideology. She has succeeded in demonstrating the interest of Lamarck's case for the history of science, even though there is much more of interest in Lamarck's thinking than she mentions. Given the important kinds of questions she has posed, and allowing that new perspectives will provide additional questions to ask about Lamarck's case in the future, there is

every likelihood that Lamarck will continue to be of considerable interest for the history of science when his next major anniversary comes around. □

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Reviews of bonding

S. R. Elliott

Electrons and Transitions. Vol. 39 of *Structure and Bonding*. Series editors J. D. Dunitz, J. B. Goodenough, P. Hemmerich et al. Pp.120. (Springer: Heidelberg and New York, 1980.) DM64,\$35.90.

VARIOUS themes are common to the different branches of chemistry; one such, underlying inorganic and physical chemistry in particular, is bonding and its concomitant structure. This is the title of a continuing series published by Springer, each book comprising a collection of articles. The latest addition is Vol. 39, subtitled *Electrons and Transitions*.

This volume contains three articles. The first, by D. W. Clack and K. D. Warren, is entitled "Metal-Ligand Bonding in 3d Sandwich Complexes". The review concentrates on the bonding between the metal and the ligand rings for various series of first-row transition metal sandwich complexes containing from three- to eight-membered carbocyclic rings. Experimental data from electronic spectra, ESR and photo-electron spectroscopy are compared with the predictions obtained principally from the authors' INDO SCF molecular-orbital calculations. The article runs to 41 pages and 80 references are included.

The second article, "Ligand Polarization Model for the Spectra of Metal Complexes: The Dynamic Coupling Transition Probabilities", is by S. F. Mason. Crystal field theory, the principal independent-systems model in which metal-ligand overlap is neglected, has been extremely successful in accounting for the character of the structure and bonding of metal complexes. Nevertheless, its shortcomings are not confined to neglect of metal-ligand electron exchange. In a first-order independent-systems treatment, either the ligands perturb the metal ion (crystal field theory) or, in the other extreme, the metal ion perturbs the ligands (ligand polarization model). This article, of 39 pages and 82 references, considers applications of the latter approach to oscillator strengths of lanthanide and transition metal complexes, rotational strengths of chiral coordination compounds and the Faraday effect terms

of achiral complexes.

The final article, by L. R. Balsenc, is "Sulfur Interaction with Metallic Surfaces and Interfaces studied by Auger Electron Spectroscopy (AES)". This sets out to review the contribution of AES to the studies of the behaviour of sulphur on and with metallic surfaces including, amongst others, gas adsorption and desorption kinetics, surface and grain boundary segregation, corrosion, passivation and catalyst poisoning. This technique has several advantages; it is very surface-sensitive and adsorption-site positions can be deduced from the angular-dependent Auger emission from single crystal substrates, and the method's high speed of data acquisition is of great use in kinetic studies. In contrast to another commonly used technique, X-ray photo-emission spectroscopy (XPS), AES is some 20 times

more sensitive for sulphur detection, although XPS is far superior in the elucidation of the chemical state of the atoms by means of the 'chemical shift' since the shift is very small compared with the Auger peak width and hence easily obscured. The review is short, only 32 pages, but contains an extensive bibliography, and will be of interest in both the academic and industrial spheres.

In summary, this is a well presented book with only one niggling detail, the use of the American spelling, sulfur, although all other usage is English; the book's appeal should lie primarily with researchers in those fields represented by the articles included. □

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Floras finished and in progress

Peter D. Moore

Flora Europaea, Vol.5. Edited by T. G. Tutin, B. H. Heywood, N. A. Burges et al. Pp. 452. (Cambridge University Press: Cambridge, UK, and New York, 1980.) £37.50, \$85. *Flora Palaestina*, Part 3. Text pp. 481, plates 757. By N. Feinbrun-Dothan. (Israel Academy of Sciences and Humanities: Jerusalem, 1979.) IL500, \$55.

"THE publication of this final volume of *Flora Europaea* represents a landmark in European floristics". So begins the preface to Vol. 5, and one can detect a flavour of relief coupled with the justifiable pride of this statement. The concept of a European flora was formed some 26 years ago and it was then hoped that it could be completed within 12 years; but the gestation period of this *magnum opus* proved far longer than was anticipated. This must make the final parturition a yet more satisfying occasion for the editorial committee.

The final volume is dedicated to the monocotyledons and it follows the same style adopted in previous volumes. The British botanist will find few surprises in

the arrangement and nomenclature; we are already accustomed to such transmutations as *Endymion* to *Hyacinthoides* and we could have predicted movements within the genus of marsh orchids, *Dactylorhiza*, *D. purpurella* and *D. praetermissa* are now regarded as subspecies of *D. majalis*. The subspecies *ericetorum* of *maculata* is absorbed into *ssp. maculata*.

These, however, are trivia. The importance of this work is the establishment of an authoritative description and definition of the flora of the whole of Europe. It is the first such account ever published and it is unlikely to be surpassed in our generation. The editors are to be admired for having devoted a large portion of their working lives to the project, and Cambridge University Press are to be congratulated on undertaking such a gargantuan publishing task and for the high standards of production. The complete set of five volumes now costs in excess of £150, but this is a wise investment for any serious botanist, amateur or professional, who wishes to be fully informed about the wealth of Europe's flora.

The development of the *Flora Europaea* project has not been an isolated process. Floras covering adjacent regions of south-west Asia are proceeding apace. Within the Soviet Union, *Flora Europaea* covers the entire area west of the Ural Mountains, to 60°E, and on the southern side of the Black Sea P.H. Davis' work *Flora of Turkey and*

the *East Aegean Islands* is continuing to develop. Publication of this series of volumes from the University of Edinburgh Press began in 1965 and still proceeds. Similarly, to the south of the Caspian, K. H. Rechinger, based in Vienna, continues to work upon *Flora Iranica*, which commenced publication in the early 1960s.

A further, well advanced project is *Flora Palaestina* which is being published by the Israel Academy of Sciences and Humanities, Jerusalem. The recently published third part of this series covers the Ericaceae to Compositae, and the geographical range of the Flora encompasses Israel and the west and east banks of the Jordan rift valley, south to Elat and Aqaba. There are keys to genera and species of Palestinian plants plus detailed descriptions of each species. These descriptions include notes concerning the ecology and habitats of the plants, as well as their geographical range within the study

area and their full world range. These are useful notes which are not generally found in *Flora Europaea* because of pressure on space.

Each part of *Flora Palaestina* is accompanied by a volume of plant drawings which are of very high artistic quality and botanical accuracy. Five artists have been employed in this task, but a commendable uniformity of style has been maintained. Since a fair proportion of the plants illustrated are widely distributed in the eastern Mediterranean, these drawings will prove useful and interesting to European botanists as well as to the Israelis. Which leads one to the question whether a similar set of illustrations could be prepared to accompany *Flora Europaea*? Herculean, perhaps, but nevertheless a profitable exercise. □

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Pore structure and flow modelling

D. J. Gunn

Porous Media. By F. A. L. Dullien. Pp. 396. (Academic: New York and London, 1980.) \$42, £23.60.

IN THE preface Professor Dullien gives the primary purpose of this book as "presenting in an organised manner the most pertinent information available on the role of pore structure and then putting it to use in the interpretation of experimental data and the results of model calculations". In pursuit of these aims the book is divided into two principal parts, the first of which is devoted to the description of pore structure, the second to flow properties of porous media.

Work on pore structure is described in two 70-page chapters. In Chapter 2, "On Capillarity in Porous Media", the description of capillary pressure measurements in porous media is followed by a description of pore network models of capillary tubes and capillary pressure functions generated by network models. In Chapter 3 porosity and permeability are defined, the measurement of pore size and pore size distribution is considered, and the measurements of pore size distribution are described for a range of materials. Single-phase flow in porous media is considered in Chapter 4 and includes a survey of some of the available correlations between flow and pressure gradient, statistical permeability models and network models, with sections on gas flow and diffusion, and non-Newtonian flow. The description of multiphase flow of immiscible fluids, Chapter 6, is mainly directed to the flow of

oil and water through both consolidated porous solids and unconsolidated solids, while the last major part of the book is Chapter 7 on miscible displacement and dispersion.

The emphasis of the book is on fundamental studies of interest in petroleum recovery and production, although topics such as the formation, flow and dispersion of micro-emulsions that are of interest in secondary recovery are not considered in detail. The book would have been more helpful if the description of models for flow and dispersion in porous media was more critical. The most important criteria for the success of a model description are that the model should be well founded, and that it should describe experimental phenomena without invoking significant further measurements to define the parameters of the model when applied to a particular situation. Some of the model descriptions of dispersion in porous media, although considered in some detail in the book, have not been shown to relate to experimental measurements of dispersion, while the use of a bivariate pore size distribution to estimate permeability requires more experimental work than a direct measurement!

However, for those who have already been introduced to the study, the publication of a research monograph on flow in porous media is timely because of the economic importance of techniques for secondary oil recovery. Further, it is very useful to have this comprehensive survey available as an aid in process development and in the mathematical modelling of field flows. □

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Macromolecules in 3D

Colin Blake

Teaching Aids for Macromolecular Structure. Compiled by Richard J. Feldmann and David H. Bing. Instructor manual pp.114 with 116 stereoslides; student kit 49 stereoslides with viewers. (Taylor-Merchant: New York, 1980.) Instructor kit \$210, student kit \$15.

THE APPLICATION of the X-ray diffraction technique to crystalline and paracrystalline biological materials has produced a solid body of information on the structure and function of many biological macromolecules. After an initial period of scepticism concerning the significance of the results, this material has now become reasonably well integrated into the broad field of biochemistry, and is now routinely taught at the undergraduate level, not only to biochemists, but also to those in other more biological or physical sciences. Teaching this material is however fraught with problems, most of which derive from the sheer complexity of biological macromolecules. Although the repetitive structures of DNA, the α -helix, β -sheet and even the collagen helix are fairly easy to visualize, the molecules of t-RNA, supercoiled DNA and most proteins and enzymes are much more difficult to illustrate. To overcome these difficulties a variety of short cuts are no doubt taken; the most extreme is to ignore all of an enzyme except its catalytic site, which at best does less than justice to enzymes and at worst gives a completely distorted view of the *raison d'être* of the subject, the attempt to understand the characteristic integration of structure and function exhibited by biological macromolecules. To say this does not necessarily imply criticism of the people who use the short cuts; it probably has much more to do with the sheer difficulty of producing teaching material that shows the extent of the structure-function integration. Even with a complete set of plastic and wire models, and mono and stereo slides there are problems: models are either too small or too complex to point out particular features to a class of students, and slides, even those in stereo, have of necessity to be simplified and cannot show all the features of a complete molecule. And, of course, many departments are not comprehensively equipped in this way.

With these problems as an overt background, a new aid for teaching macromolecular structure has just become available. Called TAMS, an acronym for Teaching Aids for Macromolecular Structure, it has been compiled by Richard Feldmann of NIH and David Bing of The Harvard Medical School. TAMS consists of a large ring-back folder containing a

students' text, a teachers' text and a collection of 116 2" x 2" colour stereo slide pairs. A selection of the slides has been included on seven large cards which together with a simple but ingenious stereo viewer are packaged in a small plastic wallet for use by individual students. Although a small stereo viewer is included in the TAMS folder the stereo slides are intended for projection to the class using two normal slide-projectors (a stereo projector may not be suitable), an aluminized screen and polarized viewing glasses. Details of the simple modifications needed to be made to the projectors and an address where the screen and glasses can be obtained are included.

The text of TAMS is essentially a straightforward description of the contents of each slide, and need not be considered further. The slides are the heart of the system and it is on their technical quality and illustrative suitability that TAMS must be considered. One must say at once that the technical quality of the slides is quite superb. The vast majority of them are computer-drawn, coloured and shaded views of space-filling models of nucleic acids, proteins and their ligand complexes, that paradoxically do not, and at the level of accuracy shown, could not exist. Nevertheless the illusion of looking at three-dimensional, coloured space-filling models of a variety of biological macromolecules is totally convincing and one that students will readily accept, certainly with enjoyment and one hopes also with understanding and insight. Technically these slides are so good that one feels rather churlish in making criticisms: but there are two aspects on which I feel some dissatisfaction. First, although the muted colours are very agreeable to look at there are some difficulties in distinguishing the colours that are described as "light charcoal" and "pale blue" but which appear as very similar shades of light blue-grey. Also, the bluish shading of white spheres and the white highlights on pale blue or even pink spheres can lead to confusion. Secondly, and more importantly, the very success of the space-filling illusion appears to have seduced the compilers into using them almost exclusively, when many more line drawings would have been very valuable in providing aids to identifying particular features in the often complex space-filling picture.

Having established the technical excellence of the slides, one now has to consider how well they illustrate the seven "themes" of TAMS: Peptide Bond, Alpha Helix, Beta Sheet, Tertiary Structure, Quaternary Structure, Prosthetic Groups and Active Sites. Here I must confess to active disappointment, particularly in the case of the student cards, with both the overall organization of the material and the way in which it is illustrated in individual slides. The first student card on the Peptide Bond gets off to a very bad start. Four of its

seven views illustrate a molecule called Synthetic Di-Alanine, with no more explicit description in either student or teacher text, but which is not the expected alanylalanine but an acetyl derivative whose additional methyl group perversely complicates the intended illustration of the spatial relationship of the methyl side chains and the peptide group. The final slide on this card shows a proline residue almost edge-on in the myoglobin molecule which thankfully illustrates rather ineffectually the text's repetition of that old misconception that prolines cannot be accommodated in helices. Why it was decided not to use this card to illustrate the Ramachandran dipeptide analysis, or why the card on Tertiary Structure does not consider the Levitt and Chothia classification of protein structure is not explained in the text, but what is shown in no way replaces these important insights into the principles of protein structure. The card on Active Sites brings out another problem — that of identifying particular residues in a complex, space-filling picture. Because no visual guide is given it is really very difficult, even when one knows what one is looking for, to identify the serine and histidine in the active sites of the serine proteinases. I can see many students giving up slides like this, and there are quite a number of them, simply because of the difficulties of identification. In contrast the other kind of slides in which specific features are picked out in particular colours can be immediately comprehended and represent the proper use of the system. One has to conclude that the primary requirement of teaching material, that it should be closely organized and absolutely clearly illustrated, has not been consistently borne in mind when TAMS was being planned. Finally, the choice of material in the cards and in the main body of the slides is not as comprehensive as it might be: there are no examples of fibrous

proteins or carbohydrates, no illustrations of viruses apart from two slides of the filamentous type, there is insufficient consideration of intracellular proteins and too much emphasis on certain small proteins, most notably parvalbumin and cytochrome *c*. Of course one is aware that the authors are dependent on information being made available from recent research, which may account in part for the restricted choice of material, but certainly not all.

I have no wish to overemphasize the deficiencies in the choice and display of the material in TAMS. There are many slides which are both a joy to look at and which illustrate important aspects of protein and nucleic acid structure that could scarcely be demonstrated in any other way. The small set of views of nucleic acid structures is particularly striking, and especially that of supercoiled DNA, while in the enzyme collection the visualization of the deep cavities in which the active sites of carbonic anhydrase and carboxypeptidase are located is most successfully carried off. Once seen, many teachers will see slides of this kind as essential and irreplaceable visual aids to their courses in macromolecular structure. For this reason TAMS must be welcomed and Feldmann and Bing commended for overcoming the considerable technical and commercial difficulties they undoubtedly faced in making these slides generally available. Nevertheless a slight sense of disappointment of an opportunity missed persists. In the second edition of TAMS, one would ask the compilers to ignore McLuhan's notorious motto "The medium is the message" which they use to preface the text, and suggest that they keep the medium but think about improving the message. □

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Polymer science and scaling

M.A. Moore

Scaling Concepts in Polymer Physics. By Pierre-Gilles de Gennes. Pp.320. (Cornell University Press: Ithaca, New York, and London, 1979.) \$38.50, £23.

OUR KNOWLEDGE of the physics of long, flexible polymer chains has been greatly extended in recent years by important developments in both experimental and theoretical techniques. On the experimental side the advent of high-flux neutron beams has made it possible to observe a labelled chain in a sea of chemically identical but unlabelled chains. This is done by replacing some of the protons of a polymer

by deuterons which scatter neutrons more intensely than protons. Extensive data on long-range conformations and correlations have now become available. A traditional tool for studying polymers in solution has been that of light scattering, which has been improved almost beyond recognition by the development of the 'photon beat' method which now allows study of the dynamics of polymer chains in the frequency range 10^6 – 10^8 cycles.

Alongside this development in experimental technique there has been a sharpening in our theoretical understanding brought about by the discovery of the close connection between polymer statistics and phase transitions. This discovery has enabled concepts useful in the study of critical phenomena such as scaling and critical exponents to be transferred to the field of polymer science. De Gennes has been the foremost worker in this area and

his book is essentially a collection of the results which he and his co-workers have obtained by the application of scaling ideas. These results have been largely confirmed by the new experimental methods and the general agreement between theory and experiment is most impressive. However, the scaling method only gives an understanding of 'universal' properties such as, for example, the molecular weight dependence of the dimensions of a flexible polymer molecule in solution. Numerical prefactors, which are specific to a given monomer, are not predicted by this approach.

In the book the scaling concept is applied to a great variety of topics. Polymers in dilute and semi-dilute solution, theta solvents and good solvents, gels, melts and the dynamics of single chains and networks are just some of the problems successfully treated. It is not easy to apply scaling ideas and much physical insight is needed to get correct results. De Gennes has such insight and he is also adept at explaining his reasoning. Further work is now needed to derive scaling results in a more systematic way, and in the last chapter de Gennes outlines the renormalization group which probably provides the best way of putting

the scaling approach on a firmer theoretical foundation.

The book is written mainly for experimentalists in polymer sciences who wish to incorporate the recent theoretical advances into their mode of thinking and for polymer theorists who want to achieve a qualitative understanding of the scaling method. It is an excellent work and no worker in the field can afford to be without a copy. □

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Applied bifurcation theory

Michael Berry

Bifurcation Theory and Applications in Scientific Disciplines. Edited by O. Gurel and O.E. Rössler. Pp.708. (New York Academy of Sciences: New York, 1979.) \$85.

THIS is a collection of over 50 papers presented at a conference held in New York in 1977. Bifurcation is sudden qualitative change in the solutions of an equation or system of equations, produced by the

smooth variation of one or more parameters. As might be expected on the basis of such a general definition the papers cover a vast range of subjects, well indicated by the titles of sections into which the book is divided: mathematics, biology, chemistry and chemical engineering, physics, ecology, economics, engineering, and experimentation and simulation. What all these subjects have in common is that they involve nonlinear equations, and the reason for the emergence of bifurcation theory as a separate discipline is that for some classes of nonlinear equations the bifurcation behaviour is beginning to be well understood.

In the simplest case of all, dynamics is ignored (i.e. the system's state is considered to change very rapidly) and bifurcation theory reduces to the classification of equilibria, accomplished by Thom's theory of catastrophes. In several papers here a refined version is applied to analyse the types of buckling that occur in elastic structures under increasing load in the presence of imperfection (of design or manufacture) described by several parameters.

When dynamics is taken into account, the variety of possible bifurcations is much greater, but the following sequence of events is quite common: as a parameter P increases, a stable equilibrium bifurcates into a stable closed orbit (Hopf bifurcation), which in turn bifurcates into one of twice the length, and so on, in a cascade of successive 'subharmonic bifurcations' accumulating at a finite value of P , beyond which the motion is aperiodic and chaotic. Such behaviour occurs, for example, in the ecological equations for the numbers of individuals in successive generations of a population reproducing in an environment with finite resources, or in the physiological control equations governing respiratory and haematological disorders, or in preturbulent fluid regimes with different Reynolds' numbers. In several papers the mathematical models give a very good description of experimental results, but when structurally stable behaviour is expected (and found) in a wide variety of contexts, it is important to be quite precise about what theory is being tested.

The general standard of exposition is high, but I cannot resist quoting one example of intolerable jargon:

The bootstrap principle of adaptability theory is: The assumption of the hierarchical and compartmental structurability of the state description of biological systems is self-justifying within the framework of adaptability theory in the sense that such structurability is a necessary condition for efficient adaptability.

I strongly recommend this book to anybody wanting an introduction to this fertile new field of applied mathematics. □

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Source book on cadmium

M.H. Martin

The Chemistry, Biochemistry and Biology of Cadmium. Edited by M. Webb. Pp.476. (Elsevier/North-Holland: Amsterdam and New York, 1980.) Dfl. 165, \$80.50.

THIS book is the second volume in a series on topics in environmental health, and consists of 11 chapters by different authors.

Most of the contributions are concerned with mammals including human beings, and indeed the editor states that the book attempts to integrate the toxicological aspects of cadmium in animals (*sensu* mammals) and human beings with the chemistry and biochemistry of the cadmium ion. The singling out of soils, plants and aquatic organisms as other environmental components to be considered serves to highlight the absence

of contributions on air, terrestrial invertebrates and non-mammalian terrestrial vertebrates. In addition the preponderance of discussion on metallothioneins (cadmium, zinc, copper and mercury), some three chapters amounting to 22% of the book and also mentioned in the aquatic context, further unbalances the presentation.

In his remarkably brief preface, scarcely half a page, the editor states that he expects that the individual chapters of the book will be read as complete entities. To this end the various articles provide useful reviews on specific topics by authoritative contributors. The various chapters are relatively up to date, most including references to some 1979 literature, although some appear to have been completed before others. In fairness to the individual authors space could have been found in the preface to mention dates of completion of the reviews.

The presentation is relatively lavish and is consistent with the Elsevier/North Holland Biomedical Press standards, with matching price. Like many books of this multi-author type, its main use is likely to be as a source book of specific aspects of cadmium biochemistry rather than as a comprehensive treatment of the environmental biology, toxicology and biochemistry of cadmium. □

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Blackwell Scientific

In their advertisement (p.xxi) in the 12th June issue of *Nature*, titles and authors of two books were transposed. *Introduction to Modern Virology*, 2nd edn, is by S.B. Primrose and N.J. Dimmock; *Pharmaceutical Microbiology*, 2nd edn, is by W.B. Hugo and A.D. Russell.

BOOKS RECEIVED

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- PAN-IAI-LIU. (ed.) Dynamic Optimization and Mathematical Economics. Mathematical Concepts and Methods in Science and Engineering-19. Pp. x + 269. ISBN-0-306-40245-9. (New York and London: Plenum Press, 1980.) \$29.50.
- WILSON, R. J. and BEINEKE, L. W. (eds) Applications of Mathematical Graph Theory. Pp. xii + 423. ISBN-0-12-757840-4. (London, New York, San Francisco: Academic Press, 1979.) £18.50 \$39.00.

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- AUDOUZE, J. and VAUCLAIR, S. An Introduction to Nuclear Astrophysics. The Formation and the Evolution of Matter in the Universe. Geophysics and Astrophysics Monographs 18. Pp. xx + 164. ISBN-90-277-1012-0. Hardback ISBN-90-277-1053-8. flexi. (Dordrecht, Boston, London: D. Reidel Publishing Company 1979.) Dfl 75 US\$39.50 hardback.
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Physics

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- DIMSDALE, J. E. (ed.) Survivors, Victims, and Perpetrators: Essays on the Nazi Holocaust. Pp. 474. ISBN 0-89116-145-7. (Hemisphere: Washington, 1980.) \$19.50.
- SIMPSON, G. G. Splendid Isolation: The Curious History of South American Mammals. Pp. 266. ISBN 0-300-02434-7. (Yale University Press: New Haven and London, 1980.) £11.00.
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- SCHREIBER, H. G. The Philosophy of Life and the Universe. Pp. xii + 169. ISBN-533-03232-6. (New York, Washington, Atlanta, Los Angeles, Chicago: Vantage Press, 1980.) \$6.50.